

nature

April 10, 1975

include that there is no justification for our actions, & that more work is needed.

The work reported in this paper was supported by the Science Research Council who paid £15,000 for a project, the title of which can only be said to bear a remote resemblance to the title of this paper. This money paid for a highly sophisticated microscope which turned out to be unnecessary, a digitiser of a type which it later transpired was possessed by many other laboratories within walking distance, a chart recorder which has been useful on a couple of occasions, a trip to America to give a ten-minute lecture at 8.20 on the first day of a conference, numerous train journeys to London to attend committee meetings the value of which the entire committee agrees privately is nil and a research assistant whose use has been minimal but whom the professor could find nobody else able to pay for.

Capital facilities were provided by the University Grants Committee and include my large office and personal laboratory which unfortunately remained empty during my sabbatical year.

I thank my research students who, initially unable to see the point of my work, are now unable to see the point of anyone else's. I appreciate their good-humoured forbearance whilst I wrote their theses for them, persuaded the examiners that this was a significant contribution to knowledge and set them up in university careers of their own despite their initial belief that they could be more productive in industry.

I thank my secretary for patiently retyping this manuscript in its entirety several times when I have discovered grammatical and punctuation errors. Her cheerfulness in re-making forty photocopies of the manuscript and dispatching them each time by air mail to my friends and enemies is acknowledged. Her skill in finding things to do during my sabbatical absence is also appreciated. Admittedly the absence was mitigated by her being able to type my book, for which I shall receive a modest royalty, and being able to look after my other private business interests for which the facilities of the department are occasionally most helpful.

I thank my wife for acting as laboratory assistant. Her initial untutored clumsiness did not deter her from continuing to try hard. She was particularly helpful as my personal assistant on my trip to America.

The computational work described in the paper was done at the University Computer Centre whose staff I

thank for their courtesy in helping me debug the program and in carrying over my paper output to me every day. They may be reassured that their work was not in vain—the final results agreed perfectly with predictions based on my pocket calculator. Although the amount of printing on each page of my output may have seemed to them somewhat slight, the paper is being put to good use for crayonning in my son's nursery school.

I thank the departmental tea-lady who never forgets to acquire, during her lunch break, a large cream bun which I particularly like to have delivered to my office with my cup of tea at four o'clock.

The equipment used in the experiments reported in this paper will not, as far as I can see, be used by me again. Nevertheless, I cannot, alas, see my way to letting anyone else use it, as it has certain characteristics which might confuse other users and there is always the possibility of damage. Likewise although the data which I have amassed is by no means completely described in this paper, I do intend some day to look once again at these results. In the meantime it would be inappropriate for anyone else to attempt to set up similar experiments; I am perfectly happy to share my unworked data with anyone. But I must point out that the information on the magnetic tapes is not self-evident, so anyone wishing to share would do well to visit my laboratory—I would happily co-author any paper that emerged from such a visit.

Finally, I must say something about this idea of accountability in science which some people are talking about these days. The thought that a researcher at the frontiers of knowledge should be spending the government's money and his own and his students' time other than in an efficient and conscientious way is quite alien to me and contrary to the spirit of academic freedom. I know I give value for money in my research and I need no small-minded bureaucrat telling me how to cut costs—besides 'value for money' is mercifully indefinable in the world of pure research. Yet, for what it's worth, I am prepared to say that I have rigorously observed the government's energy conservation measures. To conserve electricity, nobody on this project has been allowed to work in the laboratory during the hours of darkness. This has, of course, somewhat affected our productivity, but it reflects my concern that at a time like this we should not waste the nation's resources.

RECEIVED
APR 10 1975
UNIVERSITY OF CAMBRIDGE
LIBRARY





IN February 1974 we made contact with Mr Uri Geller and were able throughout the year to observe psychokinetic phenomena at four sessions, in addition to participating in sessions with children. A brief report of our observations has been prepared for circulation to those interested. Among the claims made are that plasticity of metal was paranormally produced and that part of an encapsulated single crystal of vanadium carbide apparently vanished. It is clear both to us and to the referees used by *Nature* that this account does not amount to a rigorous loop-hole-free report on a subject historically shot through with loop-holes. Nevertheless we believe that we have some significant work in progress, and the experience we have gained may be of value to other physicists interested, like ourselves, in the interactions between mind and physical systems.

We have come to realise that in this domain the experimental situation is different in certain crucial ways from that which has been common in scientific experimentation. This is because the phenomena under investigation have to be produced from the minds of one or more of those who participate. Relationships among the participants therefore play a much more essential role than is usual in traditional scientific fields. These relationships have to be taken into account in a way that is somewhat similar to that needed in the disciplines of psychology and medicine. But, of course, this does not mean that we are committed from the outset to the belief that paranormal phenomena are genuine. Rather our minds are open to all possibilities, and our wish is simply to discuss what is the truth about these phenomena. Indeed, our experiments contain features designed to clear up a number of doubts that naturally arise in this type of work. Nevertheless, in addition to being careful in this way, we have also to be sensitive and observant, and not to react with a preconceived pattern of tough-mindedness that will interfere with our perception, and that may destroy the very possibility of the phenomena that we wish to study. With proper sensitivity and perceptivity we attempt to develop an approach that will adequately allow for the interpersonal element and yet permit us to engage in valid scientific research.

One of the first things that reveals itself as one observes is that psychokinetic phenomena cannot in general be produced unless all who participate are in a relaxed state. A state of tension, fear, hostility, on the part of any of those present generally communicates itself to the whole group. The

entire process goes most easily when all those present actively want things to work well. In addition, matters seem to be greatly facilitated when the experimental arrangement is aesthetically or imaginatively appealing to the person with apparent psychokinetic powers.

We have found also that it is generally difficult to produce a predetermined set of phenomena. Although this may sometimes be done, what happens is often surprising and unexpected. We have observed that the attempt to concentrate strongly in order to obtain a desired result (the bending of a piece of metal, for example) tends to interfere with the relaxed state of mind needed to produce such phenomena. It appears that what is actually done is mainly a function of the unconscious mind, and that once the intention to do something has been firmly established, the conscious functions of the mind, in so far as they have bearing on the goal, tend to become more of a hindrance than a help. Indeed, we have sometimes found it useful at this stage to talk of or think about something not closely related to what is happening, so as to decrease the tendency to excessive conscious concentration on the intended aim of the experiment. A comparison might be made with the process of trying to go to sleep, for which is needed a firm intention, without subsequent efforts.

Many of the conditions described above are also required for fruitful research in the natural sciences. Thus, if any of those who participate in a physical experiment are tense and hostile, and do not really want the experiment to work, the chances of success are greatly diminished. Likewise, the aesthetic appeal of the experimental set-up often helps to maintain interest and enthusiasm, whereas an attitude that consistently tends to damp these latter is evidently detrimental to the whole enterprise. In the study of psychokinetic phenomena, such conditions are clearly much more important than in the natural sciences, because the person who produces these phenomena is not an instrument or a machine. Any attempt to treat him as such will almost certainly lead to failure. Rather, as indicated earlier, he must be considered to be one of the group, actively cooperating in the experiment, and not a 'subject' whose behaviour is to be observed 'from the outside' in as cold and impersonal manner as possible.

The following analogy may help to give a more orderly overall description of the phenomena in the field. Consider a person whose hand has been paralysed as a result of destruction of nervous tissue. If this person is to regain the use of his hand, he must somehow

In the past 18 months the scientific community has been confronted with renewed claims that certain people possess paranormal powers, including the ability to effect physical changes in materials. Work has proceeded in several scientific laboratories in an effort to come to terms with these apparent abilities. What sort of attitude should be adopted in attempting to verify these claims? First, J. B. Hasted, D. J. Bohm, E. W. Bastin and B. O'Regan describe their approach in recent work done at Birkbeck College, University of London. Then J. G. Taylor, of King's College, London, who is the author of a forthcoming book on his study of these phenomena, reviews the philosophy of his work.

Metal-bending child: paranormal phenomenon? (Picture: Hemel Hempstead Gazette.)

activate new nervous pathways. How he is to do this, he does not know. All he can do, with all his energy, is to feel out the possibilities of movement and to observe with great attention and alertness what movements actually take place. He cannot describe or even think about just what it is that he does in getting his hand to move. Moreover, he cannot at first produce controlled movements, which bring about consciously intended results. Rather it is clear that the contact between brain and hand is brought about almost entirely by unconscious functions of the mind, which tend to be erratic and fortuitous. Of course, if he works with sustained interest and energy, he will generally find that his movements do begin to come closer to what he intends. But it is also clear that if he is surrounded by people who are not open to the possibility that he can move his hand or who bring about a state of psychological tension through hostility, then he will be less likely to be able to sustain the interest and energy needed for learning how to move his hand.

Those who work with such a person (for example, the physiotherapist) must evidently not be committed to resisting the notion that the hand can eventually move. For the thoughts in the paralysed person's mind, and those in the minds of his colleagues, are both important factors in bringing about success. The necessary open-ness to the possibility of an ultimate result must be maintained in the minds of all concerned, while at the same time there is a healthy capacity to be tentative and free of definitive conclusions in statements about what particular results may have been achieved at a certain stage. And so reliable inferences can be made in physiotherapy, though by methods that are rather different from those used traditionally in the natural sciences.

The analogy between this field and that of psychokinetic research is fairly clear. The main difference is this: we can account for and to some extent explain the connection between the brain and the hand in the case of the paralysed person (through the nerves which link them) but we have no way either to account for or to explain the connection between the brain and the object that is moved, bent and so on, in terms of what is now known to science. If, however, we suppose that there is some at present unknown force, energy or mode of connection, then we may also suppose that psychokinetic power may function in a way that is essentially similar to the power to move the hand. Thus, one might suggest that perhaps there is an unconscious 'feeling out' of the connection. In many cases there is a visible 'feedback' that enables a person to

recognise that he has done something, and that permits him to try to go further along the same (indescribable and undefinable) lines. But there may other forms of 'feedback'. Thus, if a piece of metal can respond to the brain in an unknown way, the brain may similarly respond to the metal. By being sensitively aware of this response, the person concerned might be able to tell when something had actually happened, even though the object in question was not sensually perceptible to him.

It is important, at this point, not to insist on having a potential theoretical explanation before one will seriously consider observing the phenomena themselves. Thus, when magnetic and electrostatic effects were first observed, it was impossible to account for them in terms of the known forces, which were considered to arise only when bodies were in mechanical contact. Evidently, this did not prevent the effects from being observed. The main aim of such observation is to give rise to an orderly account of the phenomena, which is first qualitative and



then quantitative—for example, first the qualitative observation that like charges repel, unlike charges attract, and then the quantitative observation that the force is inversely proportional to the square of the distance. On the basis of such an account, current field-theoretical explanations of electromagnetic phenomena were later developed. We propose a similar approach to psychokinetic phenomena, and in our work thus far we have tried to carry it out.

In such research an attitude of mutual trust and confidence is needed; we should not treat the person with psychokinetic powers as an 'object' to be observed with suspicion. Rather, as indicated earlier, we have to look on him as one who is working with us. Consider how difficult it would be to do a physical experiment, if each person were constantly watching his col-

leagues to be sure that they did not trick him. How, then, are we to avoid the possibility of being tricked? It should be possible to design experimental arrangements which are beyond any reasonable possibility of trickery, and which magicians will generally acknowledge to be so. In the first stages of our work we did in fact present Mr Geller with several such arrangements, but these proved to be aesthetically unappealing to him. From our early failures, we learned that Mr Geller worked best when presented with many possible objects, all together on a metal surface; at least one of these objects might appeal to him sufficiently to stimulate his energies. In a later session, we had such a set-up, which included two small plastic capsules, each containing a thin disk of vanadium carbide single crystal. A clearly observable change in the disk within one of the capsules was brought about when Mr Geller held his hands near them.

In discussions with magicians we have learned that the best conditions for a conjuring trick arise when the happening significantly precedes the observation. In the above instance we believe that the conditions were such that the failure to observe and record the precise moment of change is of no importance, because there is no known way of producing this effect within the closed capsule and no possibility of substitution. For this reason we conclude that this was something that no magician could have done.

Nevertheless, we realise that conditions such as we have described here are just those in which a conjuring trick may easily be carried out. We understand also that we are not conjuring experts, so that if there should be an intention to deceive, we may be as readily fooled as any person. Moreover, there has been a great deal of public criticism, in which the possibility of such tricks has been strongly suggested. For this reason it has often been proposed that a skilled magician should be present, to help to see to it that there will be no possibility of deception.

It is in the nature of the case, however, that no such assurance can actually be given. For a skilled magician is able to exploit each new situation as it arises in a different and generally unpredictable way. The corpus of tricks is not fixed, but rather continually changes and evolves. A particular magician could therefore say at most that he knew of no tricks that could have brought about a given set of observed phenomena. Of course, if several magicians of recognised proficiency were to conclude that what was done on a certain occasion did not in-

volve any tricks, this could help create a presumption in favour of the notion that the phenomena are genuine. In principle, we would welcome help of this kind in decreasing the possibility of deception. It has been our observation, however, that magicians are often hostile to the whole purpose of this sort of investigation, so that they tend to bring about an atmosphere of tension in which little or nothing can be done. Indeed, even if some magicians were found who were not disposed in this way, it does not follow that their testimony will convince those who are hostile, since the latter can always suppose that new tricks were involved, beyond the capacity of those particular magicians to see through them. Because of all this, it seems unlikely that significant progress toward clearing up this particular question could be made by actually having magicians present at the sessions, though we have found it useful to have their help in a consultative capacity. We have learned in such consultations not to withdraw our scrutiny from the identified specimen, from the first moment when it reaches the hand of the subject until the bend occurs. We are familiar with the use of the human hair in producing small movements, with the use of mercuric salts in alcohol to corrode metals, and with the weakening in metals produced by continued bending to and fro. We recognise that there is a genuine difficulty in obtaining an adequate answer to criticisms concerning the possibility of tricks, and that a certain healthy scepticism or doubt on the part of the reader may be appropriate at this point. Indeed, it would be inappropriate if the scientific community did not at first react in such a way. We believe, however, that our approach can adequately meet this situation.

It is essential that in at least some experiments, conditions must be controlled in such a way that the possibility of deception is insignificant. Metal bending and cleavage experiments are particularly suitable for this approach. Encapsulated specimens can play an important part, although up to the present we have only been able to achieve success with one such specimen.

We feel that if similar sessions continue to be held, instances of this kind might accumulate, so that there will be no room for reasonable doubt that some new process is involved here, which cannot be accounted for or explained in terms of the laws of physics at present known. Indeed, we already feel that we have made sufficient progress toward this point to warrant further investigations along these lines. We hope to carry out more tests and to report on them when results are available. □

It is not easy to discern a sharp boundary at which scientists must stop and turn into magicians . . .

THE crucial question that any scientist has to ask himself if he wishes seriously to investigate the paranormal (spoon-bending, telepathy, clairvoyance and the like) is how to do so scientifically. There are those who claim the very elusiveness which paranormal events have in their nature precludes any such approach. But there are also persons like myself who feel that these strange phenomena are relevant to their world view and so deserve careful study. Do we necessarily have to doff the garb of scientist when satisfying our curiosity about such events?

We can say at the outset of attempting to answer this question that if the response were "yes", science would be circumscribed in a very peculiar way. No elusive phenomenon would be allowed as a suitable object of scientific enquiry; on this score quarks, for example, would be beyond the scientific pale. To avoid such absurdity we must realise that there is a continuum of levels of elusiveness. It is not easy to discern a sharp boundary at which scientists must stop and turn into magicians.

There is one feature, however, which should be present in any phenomenon to be investigated scientifically. That is repeatability, allowing the possibility of many investigators observing the event at different times. Not only does this allow the 'consensibility' to be practised which is so crucial to scientific investigation but above all it permits the phenomenon concerned to be probed ever more deeply. Only then can the event begin to be seen in terms of current understanding. Thus the two processes of validation and model building can be carried out, possibly hand in hand or almost simultaneously.

At this point the notion of repeatability for paranormal phenomena must be clarified, since it seems to mean different things to different people. It is being used here in the following sense. Suppose that an event, such as a child causing a spoon to bend, has occurred a number of times in a given environment. The bending may not happen every time the child is tested, possibly because some crucial factor in the surroundings has changed from one test to the next without anyone realising. Yet provided that in a non-zero fraction of the test situations spoon bending (or whatever it is) has occurred and enough tests have been made to feel confident that the phenomenon will continue at about the same rate then the para-

normal event will be termed repeatable.

One of the main difficulties in applying this criterion is in the specification of the precise environment in which the event will recur. There can well be psychological pressures put on the subject by the presence of particular objects or persons, and the most suitable environment has to be found by laborious trial and error. But these problems need not make one despair of obtaining repeatability. In particular subjects should not be expected to cause a paranormal event every time they are asked to, and in any surroundings. It is like expecting a particle accelerator to produce its beam of particles whatever level of vacuum there is in the accelerating tube or whatever potential is applied. The design of such a machine is so precise that it can only function under very special conditions. We are in the position of dealing with even more complicated machinery in human beings and account must be taken of that when investigating the paranormal.

In order to be able to investigate a phenomenon further, it is necessary that it can occur under suitably modified conditions. If spoon bending were only ever possible when the subject could not be directly observed during the bending process then, even if it were repeatable, a number of explanations for it, including the obvious one, would be possible. In general it is clear that conditions must be able to be chosen so that simple explanations of paranormal phenomena, especially trickery, are ruled out.

We come, then, to the clash between the eventual rigour of science and the laxity apparently needed to produce paranormal phenomena. Seances need to be held in the dark since the light of day seems to prevent anything strange occurring. This is not true of spoon bending or distant viewing, for which there is some level of repeatability in daylight. There are other phenomena, such as dowsing, which can also bear further investigation of a sort which allows various explanations to be tested.

It is this feature of allowing critical testing which is a *sine qua non* of a phenomenon for its investigation to be regarded as scientific. The range of tests used will naturally be at the discretion of the investigator, but it is in the event itself that this 'testability' resides. Not that further enquiry need prove easy, since it will involve modifying the environment in some way that may prevent the phenomenon from recurring. If any such change is too inimical to the event then clearly it is not possible to test it at all; the event will have to be regarded as a curiosity, but one which at present cannot usefully be studied scientifically.

As new tests are tried with a subject,

the phenomenon may initially disappear but then return at a later stage after several attempts. The investigator clearly requires patience in such a situation, but this must be exercised since otherwise the phenomenon remains too poorly investigated. It may be necessary to do this gradually, but somehow or other the phenomenon must be delimited precisely enough that it can be validated and that conjecture as to its possible explanation can begin. In particular, tests must be introduced which show with utmost rigour that fraud can be excluded as the cause of the events. Provided these tests are conclusive, then the phenomenon can be probed further.

An example of this is spoon bending, which has been observed by many investigators during the past two years. Some of the accounts are valuable, though not conclusive by the above criteria. Only by careful measurement of various physical parameters during the bending process, especially the pressure applied, can the phenomenon be classified as paranormal. The measurement process itself would best be done automatically, as has been arranged in some preliminary tests with positive results. Measurement of various parameters such as temperature and radiation levels of various sorts have also been attempted. In all of these attempts care has had to be taken to relax the subject as much as possible, yet always to avoid simultaneously relaxing scientific rigour.

The problem of preventing stress by the presence of the sceptical observer seems to be handled best by using as much automatic measuring apparatus as is feasible. The investigator can then devote more of his attention to the psychology of the subject than would be the case if he had to divide his time between trying to keep the subject relaxed and observing precisely what was occurring. Film or video tape have not proved as useful here as one would like, since they only provide a single view of the event. And they cannot monitor crucial variables such as pressure or magnetic field strength.

Finally we come to the sceptic in whose presence the phenomena may not occur or who has not been able to observe the events and considers them too strange ever to be possible. Such a critical attitude plays an important role in science, though over-reaction can take place if the sceptic is too emotionally involved in the event to preserve his objectivity. Even this serves the purpose of ensuring that standards of scientific rigour are preserved. But advance in the paranormal field will be held up by too sceptical an attitude. The question to be settled is what sort of evidence would be accepted by the sceptic? The answer to this clearly

depends on the particular sceptic involved, but certain general features of such an attitude can be discerned.

The most extreme sceptic is one who will never budge from his sceptical attitude, whatever is presented to him about the paranormal. Such an obdurate attitude can only be attacked by senility and death, so we turn to the slightly less sceptical. He requires to observe the phenomenon at first hand, since it seems for him such an impossible occurrence. Yet, even when he has observed it, he may claim that fraud had occurred. He must be asked to observe in situations with all possible rigour applied. Time and patience will be needed by him to obtain results; only if he can be influenced to expend them may he be able to observe under conditions which will convince him.

It has been assumed here that it would be possible to obtain conditions for observing paranormal events under which fraud is impossible. Some claim that this is not so, but this is clearly false. It is always possible to choose conditions in which trickery can be completely excluded. A piece of metal to which a pressure sensor is attached cannot be bent surreptitiously unless the sensor is inoperative. If the method of bending were non-mechanical, such as by the application of chemicals or by the use of a laser beam, careful chemical analysis or temperature measurements would indicate this. An object caused to be distorted without contact cannot have become so by normal means.

It is not possible at present for all such sceptics to be able to observe careful tests performed on the too few subjects with apparent paranormal powers. Nor does it seem that even a fraction of them will ever be able to do so. To counter such a weight of scepticism, it is necessary to consider its source in more detail. At the root of scientific scepticism of the paranormal is the fact that the events being investigated are in contradiction with present scientific understanding. At least, it is claimed that this is a fact for all paranormal events, but there are differences between different sorts of phenomena.

Firstly there are strange events which seem to be scientifically impossible, on the face of it, but which might possibly have a scientific explanation in the future provided that the present lack of knowledge in the relevant area is acknowledged. A case in point is, again, spoon bending, for which an electromagnetic explanation has recently been suggested. To test this properly requires considerable research into the interaction of especially low frequency electromagnetic radiation with various materials up to the point of their fracture. Only very recently has a new

feature of the latter been discovered in support of the hypothesis, in that transient electric and magnetic fields have been measured in association with tensile fracture of various metals. A similar hypothesis can be put forward for telepathy and distant viewing. Recent results from the group at the Stanford Research Institute (Targ and Puthoff) indicate an upper limit to bit-rate transfer which would again implicate very low frequency radiation. Since low frequency electromagnetic radiation has been shown to have effect on psychological parameters of animals, such an electromagnetic hypothesis might just be considered.

Other physical phenomena are more difficult to understand in such terms. Thus, if objects can truly be caused to disappear from closed containers then the energy required to dissolve chemical bonds is so enormous that no feasible physical explanation can be given in present terms. The phenomena of this type are not as well attested as those of spoon bending and distant viewing, so there is still a possibility that 'travelling' objects are not totally disappearing in one place and reappearing in another. If they are, however, then clearly a contradiction has occurred with current scientific understanding of the forces of nature and the principles they satisfy, especially that of conservation of energy.

Similar difficulties arise with other paranormal events such as precognition. That is also hard to put into the usual causal framework, which uses retarded potentials as solutions of the field equations for all the forces. The advanced potential solutions have been attempted in the past, though it would be strange if such advanced effects only arose when human beings were present. Naturally enough it is also necessary to consider the part which consciousness plays in these phenomena. A consistent picture of the mind as constructed from material building blocks is far more feasible than a tachyon-like picture which at present has currency (especially because of the complete lack of evidence for the existence of tachyons).

In conclusion, it would seem that paranormal events can be investigated scientifically provided the investigator uses his patience and ingenuity to the full, both with his subjects and with his colleagues. He must aim for the highest level of scientific rigour in his tests, though be prepared to spend a great deal of time before a subject can cause a paranormal event to occur in scientifically satisfactory conditions. Furthermore the investigator must attempt to make feasible models of the events. If all goes well, he will make enough progress to build his own machine to achieve the effect. □

international news

WHEN Congress put a stop to development of an American supersonic airliner five years ago, looming large in its decision was a controversial theory put forward by Harold Johnston of the University of California, Berkeley. Johnston argued that the aircraft would emit pollutants into the stratosphere which would partially destroy the ozone layer, the ultimate consequence of which would be a large increase in the amount of harsh ultraviolet radiation reaching the Earth's surface and a corresponding increase in the incidence of skin cancer in the Northern Hemisphere. The theory has been under strong attack ever since it was first put forward, but last week Johnston was vindicated by an unusually blunt report published by a committee of the National Academy of Sciences.

The report stated that operation of 300 to 400 of the Boeing supersonic aircraft then under consideration would "in all probability" have caused a 20% increase in skin cancer in the United States—that translates to about 80,000 extra cases a year. To emphasise the point, the report added that "a few additional people would probably have died each year and a few hundred people would probably have contracted skin cancer each year, for each formerly

NAS report spells trouble for Concorde

by Colin Norman, Washington

projected US SST put into service".

Those conclusions are of more than academic interest, for they almost certainly spell more political trouble for the financially beleaguered Concorde supersonic aircraft in the United States. The immediate effect of the report is likely to be to strengthen moves to deny Concorde landing rights at American airports.

The committee's chief conclusion is that a fleet of 100 Concorde or Soviet TU-144s, operating in the stratosphere for about 5 hours a day, would cause a reduction of 0.7% in the ozone layer. The resulting increase in ultraviolet radiation reaching the Earth would be about 1.4%, and that would be expected to lead to an increase in the incidence of skin cancer in the United States of about 1.4%, or 6,000 extra cases a year.

Concorde is expected to pose less of

a hazard than the scrapped Boeing SST because it will fly at lower altitudes and emit smaller amounts of the oxides of nitrogen—the chief pollutant responsible for depleting the ozone layer. But the problem is not limited solely to supersonic aircraft, the committee notes, because wide-bodied jets also fly in the lower stratosphere and inject oxides of nitrogen into it. If the subsonic fleet grows in size, and if the aircraft are designed to fly higher, as has been predicted, then it would pose just as serious a threat to health as a large fleet of Concorde.

Those effects can at least be mitigated by some costly engine modifications, however, and the committee therefore suggests that international regulations should be drafted to ensure that cleaner engines are used on aircraft flying in the stratosphere in future years. As a rough estimate, committee members suggested at a press conference last week that it would cost about \$100 million for research and development on engine redesign, and that it would take between 10 and 15 years to equip aircraft with the cleaner engines.

The committee's conclusions are essentially the same as those reached by a three-year study conducted by the Department of Transportation which

SUPersonic airliners are not the only potential violators of the ozone layer. In fact, in view of the dismal economic outlook for Concorde and the enormous costs of operating the aircraft, the SST fleet is unlikely to become large enough to represent much of a threat to human health for many years, in which case there is probably sufficient time to develop cleaner engines to guard against potential hazards. Of more immediate concern is the possibility that propellants used in aerosol spray cans—chlorofluoromethanes, which are more commonly referred to by their trade name, Freons—may already be depleting the ozone layer at an alarming rate, and there has recently been a flurry of activity in the United States to try to assess the dimensions of that hazard.

In February, the federal government announced that it has established a top-level scientific committee to examine the potential dangers from aerosol sprays, and that it hopes to have a report by mid-June. Last month, the National Academy of

Sciences also announced that it has established a committee to look into the problem, and named Dr H. S. Gutowsky, Director of the School of Chemical Sciences, University of Illinois at Urbana, as its chairman. The study is expected to be completed within a year. The academy decided to establish the committee after an *ad hoc* panel, which met under the chairmanship of Donald M. Hunten of Kitt Peak Observatory, had suggested that there is an urgent need to determine the possible danger from aerosol propellants. After that panel had made its recommendations, Hunten suggested at a press conference that, in his opinion, the injection of Freons into the atmosphere should be halted as rapidly as possible and that a moratorium on their use should be maintained until the potential dangers have been assessed.

According to some estimates, Freons may already have been responsible for depleting the ozone layer by about 1% and, if their use continues unchecked, the depletion may

increase to about 5% by the 1990s.

Another concern that has recently been expressed is that extensive operation of the space shuttle will pose a threat to the ozone layer because it will inject hydrogen chloride into the stratosphere. NASA is now studying the basis for that concern.

Finally, NASA announced last week that it will use the Copernicus Orbiting Astronomical Observatory to monitor the amount of chlorine in the stratosphere. Freons are thought to pose a threat to the ozone layer because ultraviolet radiation may break them down in the upper atmosphere, to release chlorine, which then breaks down ozone molecules. Thus, by measuring chlorine in the stratosphere over long periods of time, it should be possible to determine the extent of the threat to the ozone layer. This will be done by comparing the ultraviolet radiation from a bright star which is absorbed at the wavelength characteristic of chlorine, first when the star is high in the sky and again when it is on the horizon.

was published earlier this year. Called the Climatic Impact Assessment Project (CIAP), that study resulted in an estimate that a fleet of 100 Concorde flying in the stratosphere for 4.4 hours a day would deplete the ozone layer by about 0.4%. The two estimates are well within the uncertainty range of the calculations, and Dr Henry M. Booker, the chairman of the academy committee, suggested in a statement last week that both of them indicate that "strict measures might be needed to protect the environment from the effects of future large-scale flight operations in the stratosphere".

Yet another report on the environmental effects of supersonic aircraft is expected to be published soon by the British Meteorological Office's Committee on the Meteorological Effects of Stratospheric Aircraft (COMESA). According to pre-publication snippets provided recently by Dr R. J. Murgatroyd, COMESA's chairman, the report will conclude that 1,000 Concorde would be expected to reduce the ozone layer by about 3%.

Much has been made of the fact that all those studies predict that the effects of Concorde on the ozone layer will be much smaller than natural fluctuations—concentration of stratospheric ozone can vary by as much as 25% from day to day, for example. But the academy committee notes that aircraft flying in the stratosphere will cause a systematic reduction in ozone concentration, superimposed on the daily fluctuations.

"Remember that skin cancer is an integrated effect (of exposure to ultraviolet radiation) over a long period of time", Booker said last week, and he noted that average exposure to ultraviolet radiation seems to be the chief factor which causes the incidence of skin cancer to vary with latitude.

Because the ozone layer is much thinner at the equator than it is at the North Pole, the amount of ultraviolet radiation reaching the Earth's surface at low latitudes is much greater than at high latitudes; sure enough, geographic variations in the incidence of skin cancer are closely correlated with geographic variations in ultraviolet flux.

The upshot of those conclusions is that if either the supersonic fleet or the fleet of high-flying subsonic aircraft is increased in size, or if a new generation of supersonic airliners, designed to fly at greater altitudes, is brought into service, potentially serious health hazards may result. The academy committee therefore suggests that regulations should be drafted post-haste to ensure that cleaner engines are developed and installed. It suggests that the best forum for drafting such regulations is the International Civil Aviation Organisation, and the World Meteorological Organisation.

In exceptionally blunt language, a high level advisory committee last month expressed concern about the ordering of priorities within the much-publicised cancer research programme in the United States. In particular, the committee indicated its "astonishment" at the low priority accorded to studies on environmental carcinogenesis, and it took a swipe at the massive amounts of money that have been poured into research on possible human cancer viruses.

The committee, a special subcommittee of the National Cancer Advisory Board, said in a report delivered to the board last month that although there is "widespread recognition of the importance of environmental chemical carcinogenesis", the National Cancer Institute (NCI) seems to have given such studies a low priority in terms of expenditures. Less than 10% of the cancer budget is spent on research into the environmental causes of cancer, the committee estimates.

Those concerns are the latest in a long line of grumbles from biomedical scientists about the content and direction of the so-called war on cancer. The grumbles have mostly been concerned with the fact that the cancer programme has been soaking up funds at the expense of other areas of biomedical research, and that it has adopted a highly targeted approach which has emphasised applied rather than basic research. But in this case, the committee is arguing that an area of research dealing directly with cancer control and prevention is being relatively neglected.

The report, which resulted from two days of meetings by the subcommittee under the chairmanship of Philippe Shubik, Director of the Eppley Institute for Research in Cancer, University of Nebraska, lists a number of recommendations for increasing the research effort on environmental carcinogens. The preamble to the report describes the committee's concerns in graphic language.

"There was an obvious sense of general astonishment throughout the meetings that the National Cancer Programme does not appear to have accorded an adequate priority nor sense of urgency to the field of environmental carcinogenesis, particularly when this concerns chemical carcinogens", the committee states. Later in the report, the committee suggests that "epidemiology thus far seems to suggest that a viral etiology for most human cancers is an unlikely eventuality; in view of this, the distribution of the budget of the NCI in the area of etiology with its emphasis on viral oncology should perhaps be reconsidered; perhaps the time is ripe for a reordering of priorities or at least for an in depth examination of this basic conclusion".

But the committee recommends that "the NCI should foster the development and validation of new and innovative bioassay techniques" for detecting and identifying potential carcinogens in the environment. In particular, the committee suggests that more special centres for research on environmental carcinogenesis should be established in the United States.

logical Organisation.

Although no threat is likely to be posed to the ozone layer by the handful of Concorde now scheduled to enter service, the academy's report is likely to strengthen opposition to any operation of the aircraft in the United States. It should be noted that the report has been published at a crucial moment, for public hearings open on April 14 on a request by British Airways and Air France for permission to land Concorde at John F. Kennedy Airport in New York and Dulles International Airport in Virginia, for a small number of proving flights later this year.

Last month, the Federal Aviation Administration (FAA) tentatively recommended that such permission should be granted, but a final decision will not be made for several weeks. In an environmental impact statement on the request for landing rights, the FAA noted that Concorde's engines emit substantially greater levels of some pollutants—particularly carbon monoxide—

and create more noise than any other passenger aircraft, but it suggested that "the environmental consequence of the limited volume of Concorde operations requested are not so severe as to compel a refusal (of the request)". Opponents, of course, did not agree.

Meanwhile, a bill has been introduced into the House of Representatives by Lester Wolff, a New York Congressman, which would prevent Concorde from landing anywhere in the United States unless it meets the federal noise and pollution standards which apply to subsonic aircraft. A few days before the FAA announced its tentative recommendation to grant the request for landing rights for Concorde, the Environmental Protection Agency proposed noise standards for supersonic aircraft which would be less stringent than those for subsonic aircraft. Hearings will be held on Wolff's bill in July by the Public Works Committee and, if passed, it would effectively deny Concorde access to the United States. □

Seminar contacts under pressure

from Vera Rich, London

THE increasing pressure on the remaining members of the Voronel Sunday seminars now seems to be spreading to their contacts throughout the Soviet Union. In Tbilisi, pressure is being exerted on the Goldshtein brothers, Isai (35) and Grigorii (42), both cyberneticists and both "refusniks" since 1971, when their applications to emigrate to Israel were rejected on the grounds of "security".

After they were refused their visas, the brothers were both dismissed from their posts at the Institute of Cybernetics of the Georgian Academy of Sciences. It is reported that the director of the institute admitted in a private conversation after their dismissal that neither brother had ever been connected with secret work but that he, the director, was "afraid".

The Goldshtein brothers have maintained close contacts with the members of the Moscow seminar, especially with Aleksandr Lunts and Viktor Brailovskii, who, since Voronel's departure for Israel, have been the leading organisers. The Goldshteins had planned to visit Lunts and Brailovskii at Passover, but were prevented from doing so by the authorities. After this attempt, they were taken to their local KGB offices, questioned about their activities and warned that their files would be handed over on April 7 to the State Prosecutor in preparation for a trial. It was also intimated that the



The Goldshteins: Isai (left), Elizaveta Bykova and Grigorii

charges against them would be linked with four other trials now being prepared in the USSR. Since Lunts and Brailovskii have already been threatened with legal proceedings, it is feared that they will be involved in these other trials.

The charge against the Goldshtein brothers falls within the category of an unpublished edict of the Presidium of the Supreme Soviet of 1972. It is alleged that since they were refused permission to go to Israel, they have been slandering the USSR, and that their activities threaten the security of the USSR—a charge which carries a maximum sentence of seven years in a strict regime labour camp.

So far, no charge has been preferred

against Elizaveta Bykova Goldshtein, the wife of Isai and, like him, a physicist. Since, however, she is the author of a survey of the problems and backgrounds of refusniks and the means used to intimidate them, which has recently reached the West through *samizdat* channels, it seems not unlikely that she too could share the fate of her husband and brother-in-law.

In the week that physicist Evgenii Levich, who was associated with the Voronel seminar, was finally allowed to emigrate to Israel together with his brother, Aleksandr (an engineer), it seems ironic that two other brothers, also associated with the seminar group, should attract the renewed attentions of the KGB. □

"A major climatic change would force economic and social adjustments on a worldwide scale . . . it is not primarily the advance of a major ice sheet over our farms and cities that we must fear . . . rather, it is persistent changes of the temperature and rainfall in areas committed to agricultural use, changes in the frost content of Canadian and Siberian soils, and changes of ocean temperature in areas of high nutrient production . . . Our vulnerability to climatic change is seen to be all the more serious when we recognise that our present climate is in fact highly abnormal".

These sentiments will already be familiar to readers of *Nature*; now they come with the official blessing of the US National Academy of Sciences in a report, "Understanding Climatic Change—A program for action" prepared as part of the US contribution to the Global Atmospheric Research Program (GARP). There is little in the report that will be new to the serious student of climatic change, although a lengthy appendix surveying past

climates does pull together in one handy review a wealth of scattered information; but the Panel on Climatic Variation which has produced the report makes some far-reaching recommendations about the nature of future research:

- The immediate adoption and development of a coherent National Climatic Research Program to include
- A Climatic Data Analysis Program with the development of new climatic data-analysis facilities and
- A Climatic Index Monitoring Program to acquire the needed data with
- A Climatic Modelling and Applications Program to accelerate research on climatic variation.

The Panel sees these American efforts as best taking place within an international framework, and suggests that the period prior to 1980 "be used to develop additional scientific and technical manpower through the establishment and support of fellowships in appropriate areas of climatic research", and that the period 1980–2000 should be designated the "International

Climatic Decades" during which data should be gathered extensively on an internationally collaborative basis and regional climatic studies of anomalies of special interest carried out.

Apart from the rather leisurely time-scale mapped out (in view of the present world food situation and the changes in climate that have occurred in the past 25 years) the report is likely to be welcomed by all of those climatologists whose lonely warnings about the importance of climatic change are now provided with the seal of scientific respectability. The cost of the massive effort needed (massive by scientific standards, but small compared with, say, the development of a nuclear missile system) must make it doubtful whether a really effective programme will take place. That is in the hands of the politicians; but politicians outside the USA should also take note of this NAS report. They may decline to take appropriate action, but they cannot say any longer that they do not have the necessary information on which to base a course of action.

John Gribbin

correspondence

The NPT and the IAEA

SIR,—Your editorial on “The unloved treaty” (March 13, 1975) shows how important it is to exercise the utmost care in reaching conclusions about political as well as scientific matters. The gist of the article is that the foundations of the Non-Proliferation Treaty (NPT) “show signs of crumbling” and that it has failed “to catch on”.

It is strange to read this at a time when all the non-nuclear-weapon countries of the EEC are at the point of completing the process of ratification of the treaty, when the Government of Japan is reported to be putting the treaty to the Diet in April for discussion and ratification and indeed when many countries are seriously concerned to prevent further nuclear proliferation and to increase the effectiveness of IAEA safeguards. During the past year we have witnessed several steps taken by the main supplier countries in this direction, such as an agreement on a list of nuclear equipment and materials which will “trigger” the application of safeguards requirements for safeguards on specialised technological information and an increased interest in safeguards in countries that have previously been somewhat indifferent.

The editorial's comments on the peaceful uses of nuclear explosions are, I believe, equally inaccurate. Article V of the NPT specifies that the potential benefits of peaceful applications of nuclear explosions be made available to non-nuclear-weapon states party to the treaty “under appropriate international observation and through appropriate international procedures.” And further, “non-nuclear-weapon states party to the treaty shall be able to obtain such benefits, pursuant to a special international agreement or agreements, through an appropriate international body with adequate representation of non-nuclear-weapon states”.

When you ask “so where is the international body?” you overlook that the IAEA, with 106 member states, would clearly seem to meet the requirements set out in NPT and that there is no need to establish a new international organisation. In 1968, the United Nations Conference of non-nuclear-weapon states recommended that the agency initiates studies on its possible functions in the field of peaceful nuclear explosions (PNE), and the agency's suitability in this respect has

been specifically recognised by its own General Conference and by the United Nations' General Assembly.

Some of the major steps taken by the IAEA with respect to the responsibilities outlined in Article V are:

- In 1972, guidelines for “The international observation of PNE under the provisions of NPT and analogous provisions in other international agreements” were developed and approved by the agency's Board of Governors.

- In 1974, an advisory group developed “Procedures for the Agency to Use in Responding to Requests for PNE-Related Services”. These procedures have also been approved by the Board of Governors.

- The Agency has convened a series of technical meetings which reviewed the “state-of-the-art”. These meetings were convened in 1970, 1971, 1972 and in January 1975.

- Potential PNE Supplier States were approached in December 1974 concerning their participation in elaborating the basic conditions to be incorporated into the special agreements referred to in Article V of NPT.

- A separate “Unit for Peaceful Nuclear Explosions Services” was established within the IAEA Secretariat in January 1975.

Far from resisting “the accumulation of yet more duties for a secretariat already fully extended”, the IAEA has taken active steps to meet its obligations as the appropriate international body mentioned in Article V of the NPT. The point that is entirely missed in your editorial is that despite the extensive exchange of information that the IAEA has promoted over several years on this subject (incidentally we do not put out “fairly enthusiastic publicity” but merely publicise the statements of national experts), the equally extensive preparatory work that the IAEA has done on the legal and administrative aspects, not a single formal request for a PNE has yet been received by us. We have had a few enquiries from member states, and we have arranged some preliminary investigations, but in no case has a serious project emerged.

Finally, your conclusion that someone should have the courage at the NPT Review Conference to suggest that “the treaty be scrapped and new approaches tried” is most difficult to take seriously. The NPT and the related Test Ban Treaty are the two major achievements of post-war arms

control negotiations. What is needed is to pursue efforts to control the spread of nuclear weapons and to strengthen and supplement NPT by additional non-proliferation nuclear arms control and disarmament measures.

Yours faithfully,

SIGVARD EKLUND

*International Atomic Energy Agency,
Vienna*

Cabora Bassa hazards

SIR,—The Cabora Bassa Dam, the second barrage across the Zambezi, closed on December 5, 1974. The 171-metres high dam, situated on the Cabora Bassa rapids (the natural boundary between the Middle and Lower Zambezi), will hold back a 250 km long lake, with a mean breadth of 11 km and a maximum capacity of 6.23×10^{10} cubic metres at a retention level of 336 m AMSL. It will inundate 2,739 square km with a mean depth of 25.2 m (maximum depth 157 m). Five south bank turbines at present nearing completion each have a power capacity of 430 MW. Four more turbines planned for a future north bank station will bring the eventual power capacity to 3,870 MW.

Pre-impoundment surveys of the status of the Zambezi and some of its major tributaries in relation to the dam, began in April 1973 and included physico-chemical characteristics of the rivers (A. Hall, I. Valente and J. da Costa Martins), phytoplankton (J. C. Oliveira), fish (R. Morais), zooplankton and benthos (the author). The objective has been to provide basic data from which to monitor future changes in the valley ecosystem, occurring directly or indirectly as a result of the dam. The work has also produced the first information on the Zambezi since Kariba was closed in 1958, and has shown the effect of the combined influence of the Kariba and Kafue dams on the river in Mozambique.

In January 1974, a small team studied the probable future impact of the dam on the valley, including such problems as aquatic macrophyte infestation (expected to include *Salvinia molesta* D. S. Mitchell, *Pistia stratiotes* L. and *Eichhornia crassipes* Mart.), fisheries development, lake management, the creation of national parks and reserves and the impact of the dam on agriculture, industrial development and existing game reserves and fisheries in the Lower Zambezi Valley. During the study, the valley planning authority

engineers, Gabinete do Plano do Zambezi (GPZ) in Tete revealed that they intended to fill the lake to capacity during a period of four months, allowing less than 60 cubic metres of water to discharge daily to the gorge below the dam. In normal years, the average flow through the gorge is of the order of 2,000–3,000 cubic metres per second.

Recommendations made by the team to the GPZ and the then Portuguese and Mocambique governments included allowance of a minimum discharge from the dam during the filling phase of 400–500 cubic metres per second with the lake taking between one to two years to reach maximum capacity, thus reducing the expected detrimental effects associated with prolonged water reduction in the Lower Zambezi. The gorge below the dam, approximately 25 km long (river depth between 30–40 m), has no source of water other than the dam. Four major rivers enter the Zambezi between the gorge and the coast, 560 km downstream, namely the Luia, Revuboe, Mazoe (Luenha) and Shire, the Shire draining Lake Malawi to the north. Unpublished data supplied by the GPZ shows that these rivers add little by way of volume (though the Shire considerably alters the chemistry of the Zambezi below their confluence) and then only during the latter part of the wet season (mid-January to late March).

Accompanying a press announcement of closure (Noticias de Mocambique, 28.11.74) was the comment that the dam would be completely closed until such time as the water level reached 306.2 m AMSL (approximately three weeks after closure according to the report), after which time water would be discharged into the gorge. The lake would then fill at a slower rate. The minimum discharge recommendations were ignored. The engineers anticipated that little or no noticeable difference in river level would occur during this early filling phase due to the expected onset of the rains. This statement contradicts the river flow data supplied by the GPZ and gives no consideration to the possible long-term ecological consequences of such action. To compound the problem, local rains did not begin until two to three weeks after closure. Within seven days of closure, the river at Tete (approximately 120 km downstream from Cabora Bassa) was reported to have dropped to dry season levels. By February 8 this year, levels had dropped sufficiently to prevent operation of water supply intakes for Tete, and no discharge from the dam had taken place up to this date. At the same time, large numbers of spawning fish were stranded in the lower river and slaughtered in large numbers by local Africans.

This apparent disregard of recom-

mendations made with long-term considerations in mind bodes ill for future co-operation between ecologist and engineer and could have important long-term repercussions for the agriculture (mainly sugar cane), fisheries and game reserves of the Lower Zambezi Valley and its floodplains. Important decisions, such as the timing of artificial floods and the controlled fluctuation of water level in order to facilitate development of hydrophytes, essential for fisheries development in the new lake, must be taken in the near future. A dialogue between ecologist and engineer is essential if a balanced use of the resource is to be made.

BRYAN R. DAVIES

*Institute for Freshwater Studies,
Rhodes University, Grahamstown,
Cape Province, South Africa*

African finds

SIR,—In his article on the Leakeys' work at East Rudolf (*Nature*, February 20, 1975) Bernard Wood writes, of the Taung discovery in 1924: "It was fortunate that the remains of a fossil monkey had been recognised at the same quarry only a few months previously." In fact, fossil baboons were found at Taung as early as 1920. On 19th May 1920, a paper on seven or eight fossil baboons from Taung was read in Cape Town before the Royal Society of South Africa by S. H. Haughton, subsequently Director of the Geological Survey of the Union of South Africa, and still later, Honorary Director of the Bernard Price Institute for Palaeontological Research at the University of the Witwatersrand. Haughton's paper of 1920 was not published in full, though he made his notes available to R. A. Dart, R. Broom and J. H. S. Gear. An abstract of Haughton's communication was published in 1925. To accommodate the small baboon skulls from Taung, he had proposed to create a new species, *Papio antiquus*. However, as his paper was not published and as no type was designated, the name *P. antiquus* was both a *nomen dubium* and a *nomen non rite publicatum*. Gear recognised that the sample of cercopithecoids from Taung included two different species to which he gave the names, *P. africanus* and *P. izodi*.

Presumably, the "fossil monkey" referred to by Wood was a baboon cranium which, in 1924, one of Dart's students, Josephine Salmons, recovered from the home of E. G. Izod, a director of the Northern Lime Company. This find led Dart to request R. B. Young, then professor of geology at the Witwatersrand University and about to survey the Taung area, to speak to the Timeworks manager in the hope of obtaining more specimens.

The recovery of the Taung australopithecine skull, said by Wood to have been discovered by accident, actually occurred as follows: Mr M. de Bruyn, a miner at Taung, had been interested for many years in collecting fossilized bones. A week before Professor Young's visit, in October 1924, de Bruyn had blasted out a number of fossils. These included the brain cast of a baboon and, embedded in another fragment of breccia, a second, larger endocast; among the other rock fragments part of a lower jaw was exposed. The manager, A. E. Spiers, and de Bruyn showed the collection to Young, who selected certain pieces which Spiers mailed to Dart. It was the second, larger endocast which turned out to be that of *Australopithecus*. Another piece of rock made a perfect join with the former and from it Dart extracted the face. (There is no evidence whatever to support Bernard Wood's statement that "The child's skull was in fact identified . . . by Professor Young": indeed this would have been virtually impossible since most of the skull was totally embedded in the matrix. Only a part of the endocast was showing on the surface of one piece and the back of the lower jaw on another piece. Identification was out of the question until Dart had extracted the specimen from its matrix in time for Christmas, 1924.)

The article also stated that Taung was situated "in what was then Bechuanaland". This common mistake arose because Taung was situated in the northernmost part of the Cape Province, an area formerly known as 'British Bechuanaland' (and totally distinct from 'Bechuanaland'). But this area was in 1924 an integral part of the Union of South Africa, entirely separate from the Bechuanaland Protectorate which is today the Republic of Botswana. This old name of the northern Cape Province led several scientists and textbook writers erroneously to place Taung in 'Bechuanaland' or even 'Botswana'.

Wood's statement that "... work has been resumed recently at three of these sites" (in the Transvaal) is misleading, since Brain resumed excavation at Swartkrans in 1965, while Tobias and Hughes began excavating at Sterkfontein in 1966. Work has continued ever since, in the case of Sterkfontein without any interruption for 48 weeks a year for over eight years. Hence, although the article admirably reflects recent work in East Africa, it does scant justice to the palaeo-anthropological researches in the Transvaal over the past decade.

PHILLIP V. TOBIAS

*Department of Anatomy,
University of the Witwatersrand,
Johannesburg 2001, South Africa*

news and views

MÖSSBAUER spectroscopy has never achieved the universality of application experienced by many other spectroscopic methods, and will probably never do so. This is not due to any intrinsic difficulty in the technique itself, but rather because the number of different elements which can be studied without special facilities is small, and also because the information obtained from the spectra, although frequently crucial to the elucidation of a structure, can rarely be fully interpreted in the absence of other data about the compound concerned. But the technique possesses a number of valuable attributes which may be exploited where other analytical methods would fail, and this allows its use in systems which would otherwise pose considerable analytical problems.

The basis of Mössbauer spectroscopy is the recoilless emission and resonant absorption of gamma rays of suitable energy. The line width of this resonant process is so narrow that the effect can only be demonstrated where the emitting and absorbing atoms are isotopically similar, spectra normally being generated by the Doppler modulation of source or absorber to allow absorption measurements over a small range of photon energies.

First observed by Rudolf L. Mössbauer in 1957, the effect was rapidly found to have possibilities for the study of changes in the electronic environment of the atoms whose spectrum was measured, because the atoms' environment influenced the nuclear energy levels between which the resonant gamma ray transitions occurred. This influence was twofold, and allowed structural information regarding the atomic environment to be obtained.

First, any difference between the s-electron density as experienced by the emitting and absorbing nuclei will cause a displacement of the absorption line known as the 'isomer shift'. This allows the comparison of s-electron densities in different compounds and thus gives, among other things, information about oxidation states. Second, any electron distribution which is not spherically symmetrical may give rise to a 'quadrupole splitting' in which the single absorption line becomes a doublet centering on the original position. The separation of the doublet is proportional to the field asymmetry, and provides information on the geometry of the atom's immediate environment. In addition, the presence

of an internal or external magnetic field may lift the nuclear spin degeneracy and be exhibited as a multi-line spectrum.

The letter from Hedges in this issue of *Nature* (page 501) exemplifies the situation where Mössbauer spectro-

Mössbauer spectroscopy in archaeology

from Nigel J. Seeley

scopy has been used to gain information which would not readily be obtained by other methods. Archaeological materials and works of art present many difficulties to the analyst. They are frequently complex and unknown mixtures, and in many cases sampling cannot be allowed. Provided that the sample geometry is suitable, wholly non-destructive analysis is possible, but unless surface scattering spectra are to be obtained it is generally more convenient to take a sample unless the object is thin, as in the case of a painting.

Also, because the Mössbauer spectrum obtained is that of one specific atomic species, the presence of impurities containing other elements, even when present as a major constituent, will not complicate the spectrum, although their attenuation of the gamma rays may be inconvenient. This means that the spectrum obtained will be only that of the atomic species being studied, and if that element is present in two or more compounds or environments in the sample

a simple and resolvable additive spectrum will be obtained.

The elements most suited to Mössbauer studies on archaeological material are iron and tin, although nickel, zinc and gold might be useful in certain circumstances.

Iron is not only a particularly convenient element for study, but its very wide occurrence in raw materials results in its presence in a diversity of artefacts. Iron impurities in ceramics were first investigated by Cousins and Dharmawardena (*Nature*, **223**, 732; 1969) for typing purposes, and at the recent Symposium on Archaeometry and Archaeological Prospection at Oxford (19–22 March, 1975) Longworth and Warren presented data relating to the black slip on certain samples of Etruscan pottery. Here the problem is to identify an iron compound present, with other minerals, in particles too small for conventional X-ray diffraction, but presenting no difficulty regarding the Mössbauer spectrum. Though it is not possible to arrive at an identification of an unknown compound from the Mössbauer spectrum alone, comparison with spectra of known likely compounds can readily be used to characterise such materials. Glasses and glazes are another class of substance which, having no long-range ordering, are unsuitable for X-ray diffraction, but yield valuable information about the environment of iron atoms present in the network or as microcrystalline impurities.

Mössbauer spectroscopy has also been used by Keisch (*Archaeometry*, **15**, 79; 1973) to identify iron-containing pigments on paintings where its non-destructive nature and ability to 'ignore' compounds not containing iron were used to the full. Quantitative analysis using Mössbauer spectroscopy is more difficult, at the lattice dynamics of different compounds cause the absorbing atom to vary in its efficiency, but with suitable calibration a degree of quantification can be achieved.

There is therefore a number of situations where Mössbauer spectroscopy provides information on archaeological and art materials which is otherwise difficult to obtain, and much useful work remains to be done on glazes and colouring materials. Ancient alloys have been little studied from this point of view, and it is likely that such materials as tin bronzes would repay investigation.

Nuclear incompressibility

from P. E. Hodgson

MANY different measurements of the sizes of atomic nuclei have shown that their volumes are closely proportional to the number of nucleons they contain. It is thus a very good approximation to assume that the nuclear radius is proportional to $A^{1/3}$, and indeed the relation $R = 1.25 A^{1/3} \times 10^{-13}$ cm is widely used. This result may also be expressed by saying that nuclear matter is incompressible, so that the addition of more nucleons to a nucleus does not increase its density but only its size.

But nuclear matter is not infinitely incompressible, and several lines of evidence enable us to estimate its compressibility. None of these is very direct, so there is still considerable uncertainty in the values obtained.

In classical physics, incompressibility is measured by the energy needed to reduce the volume (or the density) by a definite amount. Since at equilibrium the rate of change of energy with density is zero it is usual to define the incompressibility K by the second differential evaluated at equilibrium radius R :

$$K = R^2 d^2\varepsilon/dr^2$$

evaluated at $r=R=r_0A^{1/3}$, where ε is the binding energy per nucleon.

Using the semi-empirical mass formula and neglecting derivatives of symmetry terms gives

$$K = K_M + K_S A^{-1/3} + 6e^2 Z^2 / 5r_0 A^{4/3}$$

where K_M is the nuclear matter incompressibility and K_S the nuclear surface incompressibility. This shows that the nuclear incompressibility is a function of A .

The incompressibility of nuclei can be estimated indirectly in two ways, one depending on our knowledge of the effective nucleon-nucleon forces and the other on the identification of compression oscillations.

One of the most widely used nuclear theories derives from the Hartree self-consistent field theory for atoms. In this theory each electron is treated as moving in the field of the nucleus and of all the other electrons. If a reasonable estimate of this field is made, it can be used in the Schrodinger equation to calculate the wave functions of the electrons, and hence to give a more accurate estimate of their field at any point. This calculation is continued until it converges, so that the field coming from the solution of the Schrodinger equation is the same as the field used to solve the equation.

Such calculations have been made for nuclei, with appropriate modifications to take account of the absence of a central

particle, and the equations are usually solved by electronic computers. The complete calculation from the nucleon-nucleon interaction is very complicated, so simpler methods have been developed using effective interactions. One of the most successful of these is the Skyrme interaction, and Vautherin and Brink (*Phys. Rev.*, **C5**, 626; 1972) have used it to calculate a wide range of nuclear properties from the nuclear Hartree equations. The charge and matter distributions of a range of nuclei, together with the energies of their single-particle states are given very well, so it is perhaps reasonable to accept the values of other nuclear properties derived from the same interaction, even though they cannot be verified independently.

Among these properties is the nuclear incompressibility, and the calculations of Vautherin and Brink gave values of 370 and 342 MeV for the nuclear matter incompressibility calculated from two of their most successful interactions. Earlier nuclear matter calculations by Brueckner and Gammel (*Phys. Rev.*, **109**, 1023; 1958) starting from a phenomenological form of the nucleon-nucleon interaction gave $K = 187, 167$ and 172 MeV for three different interactions, while Campi and Sprung (*Nuclear Physics*, **A194**, 401; 1972) found $K_M = 190, 213$ and 295 for three more forces. Statistical calculations by Stocker (*Nuclear Physics*, **A166**, 205; 1971) gave $K = 180, 230, 195$ for three different forces.

Pandharipande (*Phys. Lett.*, **31B**, 635; 1970) has calculated incompressibilities for a range of nuclei using the constrained Thomas-Fermi method and finds values ranging from $K = 123$ MeV for ^{40}Ca and 176 MeV for ^{120}Sn to 190 MeV for ^{208}Pb . These values are given by the above expression if $K_M = 266$ MeV and $K_S = -496$ MeV.

These calculations with nuclear forces carefully adjusted to fit certain nuclear properties give values of the nuclear matter incompressibility that vary in the range 150–400 MeV. This indicates that the incompressibility depends on aspects of the nucleon-nucleon interaction that do not strongly affect the more easily measurable properties of nuclei, so it is difficult to know the reliability of the results obtained.

Another approach to the problem of determining the nuclear incompressibility makes use of a particular kind of nuclear oscillation called a 'breathing oscillation'. The more familiar types of nuclear vibrations are shape vibrations, like those of a liquid drop or a jelly, and these can take place in an incompressible medium. But if the medium is compressible, however slightly, it can oscillate simply by changing its size, while remaining all the time spherical. It would look as if it were breathing and hence the name 'breathing mode' for these oscillations.

The higher the incompressibility, the

higher the energy of the excited nuclear state corresponding to such an oscillation. Using a hydrodynamical model (Walecka, *Phys. Rev.*, **126**, 653; 1962) the energy is given in terms of the incompressibility by

$$E \approx (\hbar^2 K / m r_{\text{rms}}^2)^{1/2} \approx 6.9 K^{1/2} A^{-1/3}$$

(Pandharipande, *Phys. Lett.*, **31B**, 635; 1970).

It is not easy to identify such states in nuclei. They have a monopole vibrational character and so have spin and parity 0^+ and strengths that exhaust a large fraction of the EO sum rule. Such states should be readily excited by reactions like inelastic electron scattering. One such state was reported by Pittham and colleagues (*Phys. Rev. Lett.*, **33**, 849; 1974) at 8.9 MeV in Pb, but this corresponds to a rather low incompressibility of $K \approx 90$ MeV, so this identification remains uncertain (*Nature*, **253**, 92; 1975).

In oxygen detailed calculations by Sharp and Zamick (*Nuclear Physics*, **A223**, 333; 1974) gave about 28.5 MeV as the energy of the breathing mode state, but it is not yet possible to identify this with an actual state. The difficulty of finding the breathing mode states thus prevents them being used to determine the incompressibility at the present time, but the development of more specific ways of exciting these states may eventually make this possible.

Breakdown of superfluidity

from a Correspondent

WHAT determines the maximum velocity with which superfluid helium will flow without friction? Can we understand the dissipative processes that occur at supercritical velocities? These are fundamental questions that low-temperature physicists have been asking ever since Landau published his classic theory of the mechanism of superfluidity (*Fiz. Zh.*, **5**, 71; 1941 and **11**, 91; 1947). A recent paper by Phillips and McClintock (*Phys. Rev. Lett.*, **33**, 1468; 1974), describing observations of the drag on an ion moving at high speed through helium, shows that we still do not have complete answers.

The liquid phase of the common isotope of helium, ^4He , becomes a superfluid when it is cooled below a temperature of about 2 K. The superfluid transition is associated with a form of 'Bose condensation' in the liquid, in which an appreciable fraction of the atoms accumulate in one particular single-particle quantum state (so that all the condensed atoms have, for example, exactly the same momentum). At a finite temperature the superfluid phase behaves like a mixture of two fluids: a 'normal' component, behaving



A hundred years ago

IN connection with the recent meeting of the French learned societies, Mr G. J. Symons writes from Paris as follows:—"M. Michel threw out a suggestion which appears to me likely to, or at any rate possibly may, be the means of averting the principal source of danger in crossing the Atlantic. I refer, of course, to icebergs in foggy weather and the total wrecks which occur from running on to them. It is well known that the proximity of icebergs is indicated by a diminution in the temperature of the sea. M. Michel's proposal is very simple: it is merely that Transatlantic steamers should carry a submerged electric thermometer, which might easily be arranged to ring a bell in any part of the vessel on the occurrence of whatever change of temperature might be decided upon." from *Nature*, **11**, 476; April 15, 1875

like an ordinary viscous liquid and composed of thermal excitations in the liquid; and a 'superfluid' component, formed from the background liquid and closely associated with the atoms in the Bose condensate.

Suppose that superfluid helium is suddenly set into fairly slow motion along a tube. The thermal excitations rapidly come into equilibrium with the tube, and this is observed as a viscous slowing up of the normal component. But the velocity of the superfluid component does not change. This velocity is identified with the velocity of the condensate; any change in this velocity would imply a change in the velocity of all the atoms in the condensate simultaneously, and this process is believed to occur with negligible probability. The superfluid component is therefore observed to flow without friction.

Similar considerations apply to the motion of an object through superfluid helium. At low speeds it suffers a drag from only the normal fluid. Its effect on the condensate is to produce only an adiabatic deformation of the condensate wave friction, corresponding to frictionless potential flow of the superfluid component past the object. At very low temperatures, when there is practically no normal fluid, the object suffers negligible total drag.

This absence of drag is expected to apply only at sufficiently low velocities. As was pointed out, in effect, by Landau, it is energetically favourable for an object that is moving at a velo-

city exceeding a certain ('Landau') critical velocity to create a 'roton', which is one of the possible thermal excitations in the liquid. In 1970 E. H. Takken published a theory of the rate at which rotons can be produced in this way (*Phys. Rev.*, **A1**, 1220), and showed that an extremely large drag should set in above the Landau critical velocity.

In practice the Landau critical velocity for the creation of rotons is hardly ever observed, since another process, the creation of quantised superfluid vortices, usually sets in at a lower velocity. The creation of these vortices raises many interesting questions, which have not all been answered, but that is another story. The one situation where rotons do seem to be created was discovered by Rayfield (*Phys. Rev. Lett.*, **16**, 934; 1966; *Phys. Rev.*, **168**, 222; 1968), who showed that negative ions in helium at a high pressure can be accelerated to a limiting velocity that seems to be equal to the critical velocity for roton creation (about 50 m s^{-1}).

It is the drag on a moving negative ion at high pressures that has been investigated in detail by Phillips and McClintock. They have extended the earlier measurements by Rayfield and others, and they have measured the velocity with which the ions move in a very high electric field. They find that the drag on an ion moving at a velocity greater than the roton critical velocity is much less than was predicted by Takken, and they also find some unexpected features in the way in which vortex creation competes with roton creation in the drag process. It seems clear that Takken's theory is inadequate, and that we have another example of the fact that even after over thirty years of theoretical effort our understanding of critical velocities in superfluid helium is still far from complete.

Lunar duststorms

from David W. Hughes

ONE piece of apparatus left on the lunar surface by the Apollo 17 astronauts was a three-axis microparticle detector. This was designed to study the cosmic dust environment at the lunar surface to determine the nature and extent of luna ejecta produced by meteorite impacts at other spots on the Moon, to measure possible increases in flux produced by Earth focusing and to look for possible interstellar particles. Berg, Richardson, Rhee and Auer (Goddard Space Flight Center, Greenbelt, Maryland) present preliminary results from this experiment in their recent letter to *Geophysical Research Letters* (**1**, 289; 1974). The sensors are

on three sides of a cube, one looking up, the other two looking along the lunar surface, pointing 25° north of east and 25° south of west respectively. The cube stands on four legs and is about 20 cm above the level of the lunar soil. The mass threshold is typically 10^{-13} g . The apparatus is on for 76% of the time, being switched off at the height of lunar day. 1,117 particles have been so far recorded during 203 days of observation.

One fascinating result from the analysis is that the particle event rate increases when the terminator (the day-night dividing line) passes over the apparatus. This increase starts some 40 h before sunrise and ends about 30 h after, the event rate typically increasing by a factor of 100 over the 'non-terminator' rate. The sunset effect is less consistent in time of appearance, duration and quality but is definitely present. Berg *et al.* rule out the direct effect of solar electromagnetic radiation as a cause because the event rate goes up long before the Sun illuminates the lunar region under consideration. Particles emanating directly from the Sun can also be discounted because the phenomenon always ends when the Sun is still rising in the sky. Rigorous thermal testing of the apparatus prior to launch also rules out temperature effects. The authors conclude that they are observing lunar surface particles, moving westward at sunrise and eastward at sunset, just as if a duststorm was sweeping across the lunar surface. This storm is along the terminator line, the dust particle moving always in the direction away from the Sun.

This could be a perfect example of electrostatic particle levitation and subsequent horizontal soil transport. It has been shown that solar ultraviolet radiation will raise the surface of the Moon to a potential of 20 to 40 volts and will give rise to a layer of high-electron density and electric field intensity that extends several centimetres above the lunar surface. This field is of the order of 60 V m^{-1} and can easily support small positively charged dust particles. Moving them bodily from place to place is another problem which requires some circumstance such as deviation of the potential of dust grains from the median value, this leading to the build-up of repulsive and attractive forces.

Dust movement could also be due to variations in the electrical surface charge with position on the lunar surface. This idea was first put forward by Criswell (*Geochim. Cosmochim. Acta.*, **3**, 2671; 1972) who suggested that large differences in the electrical surface charge could build up across light-dark boundaries due to the highly resistive nature of the lunar surface. If this is the cause of the duststorms the

horizontal variability of the electrical field as a function of solar zenith angle (or distance perpendicular to the terminator) could be inferred from the time variability of impact events obtain from the data of Berg *et al.* The electrical charge of the lunar surface, positive in the Sun and negative out of the Sun, is confirmed by the transport direction of the positively charged dust particles.

Confirmation of dust churning in the terminator zone has been obtained from Surveyor spacecraft observations of lunar horizon glow. Surveyors 5, 6 and 7 clearly detected a line of light along the lunar western horizon immediately following sunset. These observations have been discussed by Rennilson (California Institute of Technology, Pasadena) and Criswell (Lunar Science Institute, Houston) in a recent paper in *The Moon* (10, 121; 1974).

They suggest that the horizon glow is sunlight which has been forward scattered by dust grains ($\sim 10 \mu\text{m}$ in diameter, mass $\sim 10^{-13}\text{g}$, ~ 50 grains cm^{-2}) present in a tenuous cloud formed temporarily (duration about 3 h) just above the sharp sunlight-shadow boundary in the terminator zone. The grains are thought to be levitated by an electrostatic field of greater than 500 V cm^{-1} across the boundary. Rennilson and Criswell also find that the electrostatic dust motion totally dominates the churning produced by micrometeoritic impact. The scale height of the glow is between 20 and 30 cm which is of the same order as the height of the micrometeoroid detector at the Apollo 17 site.

Rennilson and Criswell comment on the fact that no obvious patterns had been seen by astronauts in the lunar soil, patterns which one would expect to result from the surface dust motion required to produce horizon glow. But as horizon glow regions constitute only a small fraction of the lunar surface and tend to occur on slopes (regions avoided by astronauts) this is not too surprising. Interestingly the Taurus Littrow region of the Apollo 17 dust experiment is surrounded by mountainous terrain which is ideal for horizon glow production. The difference between the duration of horizon glow (3 h) and the micrometeoroid dust storm (70 h) could be entirely due to the different particle sizes responsible, the 10^{-13}g micrometeoroids remaining in the lunar atmosphere much longer than the 10^{-9}g particles responsible for light scattering.

It will be most interesting to see if the more detailed analysis of the Apollo 17 site micrometeoroid data reveals storm duration to be particle size dependent. When the dust storm had died down the sensor fluxes were found to be notably similar to those

measured in deep space ($\sim 4 \times 10^{-4}$ particles $\text{m}^{-2} \text{s}^{-1}$ ($2\pi \text{ sr}$) $^{-1}$). This indicates that there is very little normal lunar ejecta with speeds in excess of 0.8 km s^{-1} , the threshold speed of the detectors. But the speed of the lunar dust storm particles must exceed this value to have been detected at all, so a certain percentage of them should have speeds in excess of the lunar escape velocity of 2.4 km s^{-1} and should subsequently leave the Moon to join the Solar System dust cloud.

Function for *E. coli* penicillin-binding protein

from H. R. Perkins

NEARLY thirty years ago Duguid proposed that penicillin caused the death of growing bacteria by interfering with the synthesis of their wall components, although at that time the composition and structure of the walls were unknown. Since then, as more detailed information about the constitution of bacterial walls has accumulated, progressively more sophisticated explanations of the mode of action of penicillin have become possible.

The walls of all penicillin-sensitive organisms contain a structural component called peptidoglycan, which consists of glycan chains of alternating residues of N-acetylglucosamine and its 3-O-D-lactyl ether, N-acetylmuramic acid, to the carboxyl groups of which are joined short peptide chains consisting of alternating L- and D-amino acids. An essential feature is that some of these amino acids are trifunctional (for example L-lysine, *meso*-diaminopimelic acid, or D-glutamic acid) and in the final peptidoglycan, which forms a retaining network round the cell, these amino acids are involved in cross-linking the peptide side chains of one strand of glycan to those of another. By 1965 the final cross-linking step of peptidoglycan synthesis had been pinpointed as the target of penicillin action. Various cell-free transpeptidation systems are now known to be sensitive to penicillin, and indeed purified soluble *Streptomyces* transpeptidases are inactivated by taking up just one molecule of penicillin or cephalosporin per protein molecule.

The amidinopenicillanic acid derivative (FL 1060) described by Lund and Tybring in 1972 acts differently from the β -lactam antibiotics previously known. In the first place it is much more active on Gram negative than on Gram positive bacteria, which is the reverse of the situation observed with most penicillins and cephalosporins.

Growth of *Escherichia coli*, for instance, is inhibited by $0.02 \mu\text{g ml}^{-1}$, and the organism does not respond to this low concentration by developing filamentous forms or 'rabbit's ear' outgrowths that lead to spheroplast production as in the classical response to penicillin. On the contrary, the shape of *E. coli* treated with FL 1060 is characteristically ovoid, and after a period of time lysis ensues without prior spheroplast formation. The second major difference is that even high concentrations of this antibiotic have no inhibitory action on peptidoglycan transpeptidation. It seems, therefore, that there must be another point of intervention for FL 1060 apart from peptidoglycan cross-linking, although the change of shape and lack of cell division in *E. coli* suggest some connection with cell wall metabolism.

The walls of Gram negative bacteria differ from those of Gram positive species in having a much thinner peptidoglycan layer and outside that an outer membrane, which contains lipoprotein and lipopolysaccharide. As Braun has shown, a proportion of one particular species of lipoprotein molecule is covalently attached to the peptidoglycan by a peptide linkage. Since this linkage is formed after the initial synthesis of peptidoglycan, it may well also involve a transpeptidation reaction that is sensitive to FL 1060, as Park and Burman already speculated.

The work of Spratt and Pardee (this issue of *Nature*, page 516) throws new light on the mode of action of amidinopenicillanic acid, and perhaps at the same time on a hitherto unspecified process in cell growth and division. They find that in *E. coli* all the six binding proteins for radioactively labelled penicillin are in the cytoplasmic membrane. Pretreatment of cells with FL 1060 at $5 \mu\text{g ml}^{-1}$ results in complete blocking of subsequent binding of ^{14}C -benzylpenicillin to only one of the six distinguishable binding proteins and even below the minimum concentration inhibitory for bacterial growth there is considerable competition for binding sites. Of a range of penicillins and cephalosporins studied, only the penicillin nucleus 6-amidinopenicillanic acid produces the ovoid cells characteristic of the action of FL 1060 and this substance also prevents binding of labelled penicillin to the same specific membrane protein, though to a lesser extent it also decreases binding to some of the other bands.

The apparent molecular weight of the specific binding protein is 66,000, and it accounts for only 0.5% of the total binding of ^{14}C -benzylpenicillin. Spratt and Pardee reasonably propose that this protein is the target by which the amidinopenicillanic acid affects the

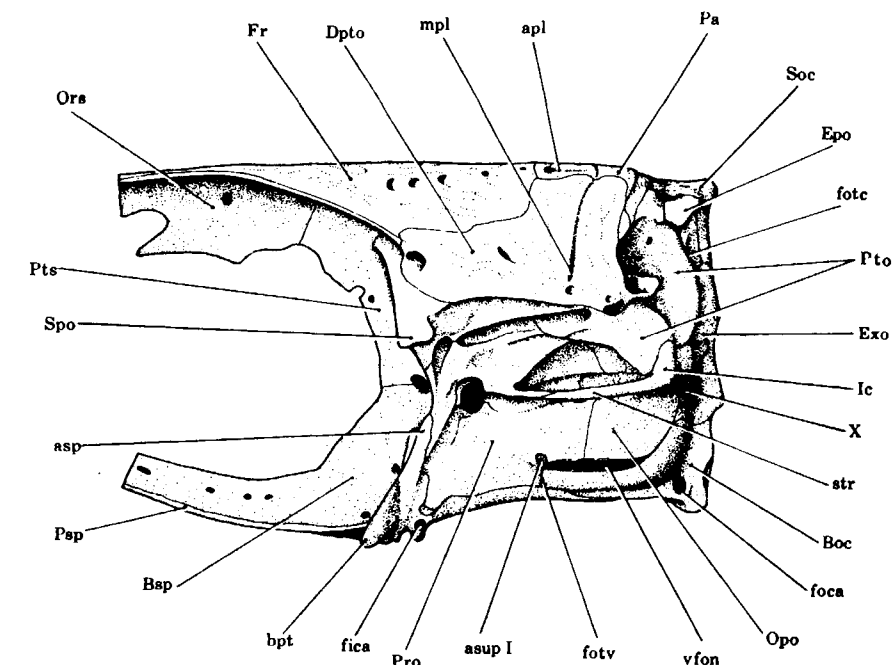
shape of *E. coli*, and that it is an enzyme involved in the terminal stages of peptidoglycan metabolism, which may be specific to Gram negative organisms.

There have been many studies of the binding of penicillin to bacterial membranes. In bacilli certain carboxypeptidases have been shown to be responsible for some of the observed binding, but many protein species are affected, just as in the absorption of benzylpenicillin by *E. coli*. The site of emergence of potential spheroplasts from *E. coli* under the influence of benzylpenicillin was used by Donachie and Begg in their studies on cell growth, and the relationship of 'penicillin-sensitive' sites to the growing bacterium was established. It will be interesting to see where, in fact, FL 1060 is absorbed to cells, but presumably this will have to await the availability of the tritiated antibiotic. The intriguing similarity of 6-aminopenicillanic acid action suggests that perhaps the binding site requires a positively charged group on the β -lactam molecule, unless, of course, the amidinopenicillanic acid is specifically taken up by the enzyme and then hydrolysed to 6-aminopenicillanic acid. The fact that the membrane protein specific for the binding of amidinopenicillanic acid also takes up penicillin implies that the majority of β -lactam antibiotics will probably inhibit its cellular function too; presumably that specific action is masked by the more far-reaching effects on peptidoglycan transpeptidases that these substances also exert. In any event, the specific action of FL 1060 should add to the already impressive list of antibiotics that have furthered our understanding of cellular processes.

Teleost ancestry, or naming of parts

from B. G. Gardiner

COMPARATIVE biology is concerned with evolutionary theory and the history of life. It is within the second domain that Colin Patterson's monumental paper "The braincase of pholidophorid and leptolepid fishes, with a review of the actinopterygian braincase" (*Phil. Trans. R. Soc.*, **269**, 305; 1975) falls. In the past hundred years there have been many great comparative works on fishes, notably those of Allis, Jarvik, Nielsen, Stensiö and Woodward. The use of Hennig's theoretical (phylogenetic) framework however has allowed Patterson to reach more clearly defined objectives than envisaged by his predecessors; while in carrying through the necessary rigorous com-



Patterson's restoration of the neurocranium and attached dermal bones of *Pholidophorus bechi* (from *Phil. Tran. R. Soc.*, **269**; 1975).

parative work he has achieved a breadth of study which sets a new standard for the future.

Palaeontologists in the past have frequently set out to answer different questions about the history of life. In particular they have searched for ancestors and have tried to reconstruct series of ancestor-descendant relationships (for example Chondrostea-Holostea-Teleost). Patterson rejects this approach as unprofitable since it has led to a fruitless search for confirmation of hypotheses. Instead his paper shows the great value of the Hennigian framework, which approaches phylogeny through a direct comparison of present-day forms, looking for collateral descendants with a common ancestor (which remains hypothetical). In this way the investigator can formulate testable hypotheses in response to precisely stated problems.

Patterson's paper provides the first comprehensive account of the Mesozoic pholidophorid and leptolepid fishes, which are of outstanding interest because they stand in the same relation to living teleosts as do their contemporaries the late Triassic and Jurassic mammals and 'quasi-mammals', to living mammals. The morphological series he describes illuminates many problems of actinopterygian evolution and ancestry. As well as clearing up the history of the cranial fissure and the ossification pattern of the actinopterygian braincase and snout, Patterson confirms the homology between the spiracular groove of primitive actinopterygians (the group

which includes most present-day bony fishes) and the 'prespiracular groove' of rhipidistians (an entirely fossil group). In so doing he rejects the hypothesis that the intercranial joint of rhipidistians and coelacanth is a primitive one related to those between vertebrae and confirms the view of T. H. Huxley in his Croonian lecture of 1858 in which he said, "It is no more true that the adult skull is a modified vertebral column, than it would be to affirm that the vertebral column is a modified skull".

Patterson has been meticulous in naming of parts, that is in tracing homologous structures throughout his series of fishes and naming them accordingly. But, like Huxley, he has realised that the possession of common structures alone does not necessarily lend support to the evolutionary hypothesis unless such structures can be deemed to be 'shared specialisations' of unique origin—found nowhere except in the species under consideration. It is in this context that Patterson has analysed the ossification of the braincase and the closure of the cranial fissure with the concomitant displacement of the pterotic by the epioccipital and the loss of the opisthotic and the endochondral portion of the intercalar. The phylogenetic homologies he has demonstrated show the dangers of the topographic approach, which assumes that a bone in a particular position in a series must always be the same bone.

Although there are no practical limits to comparative studies, and further development may be confidently predicted, it is fair to conclude that in

a hundred years time Patterson's work will stand much as it does now, a milestone in comparative palaeontology.

Palaeontologists keenly anticipate parts 2 and 3 of this work, which Patterson promises on the palate, lower jaws, gills, shoulder girdles and remaining dermal bones.

Icelandic layer 3 identified?

from Peter J. Smith

THE upwelling of magma to form new lithosphere at oceanic ridges is easy to envisage in general terms, but lack of access prevents any direct investigation of the detailed processes involved. In the study of constructive plate margins, the existence of subaerial conditions such as those of Iceland is therefore invaluable, even though by definition such environments are not entirely typical of oceanic ridge zones. Indeed, insofar as it may also lie above a rising mantle plume, Iceland itself may be even more atypical than it was considered to be in the days when only a single magma source was envisaged. But be that as it may, the few land masses astride ridges offer a unique opportunity of studying an important plate tectonic feature using more traditional geological techniques.

G. P. L. Walker began his extensive field studies in Iceland long before any of these ideas were conceived, let alone widely accepted; and over the years he has been able to throw much valuable light on the island's remarkable characteristics. But that much still remains to be learned is well illustrated by his latest report (*J. Geol. Soc.*, **131**, 143; 1975) in which he draws attention to the significance of a striking geological feature which has until now been largely ignored—the remarkable swarm of inclined basic intrusive sheets which cuts the Tertiary and early Quaternary volcanic pile along the south-east coast.

The swarm is impressive by any standards, being exposed over a zone of about 1,700 km². Over a third of that area the sheets comprise more than 10% of the total rock, and over their 100 km² of greatest concentration they account for more than 80% of the total. In all, there are tens of thousands of individual sheets with typical thicknesses of 0.2–2.0 m, chilled margins and thickness-related grain size (the thinnest sheets are basaltic, the thickest gabbroic). They increase rapidly in number down the dip, taking the concentration from less than 1% of the total rock at the top to more than 50% within a drop of only a few hundred metres. Moreover, the dip angle is itself

Oceanic polarity test fails

from Peter J. Smith

IN view of the critical roles of magnetic anomalies and the geomagnetic polarity-time scale as evidence for seafloor spreading, it would be desirable to measure the magnetic polarities of as many oceanic rocks as possible. Unfortunately, most of the igneous rocks recovered so far from the ocean floor have been unoriented dredge samples. In some cases the tops of pillow lavas may be recognised; and this limited orientation is sufficient to allow polarity to be determined by conventional palaeomagnetic methods. But for the majority of samples for which this procedure is not possible, Irving (*Can. J. Earth Sci.*, **5**, 1319; 1968) devised a method of determining polarity which involved comparison of the coercive force spectrum of a rock's natural remanent magnetisation with that of an artificially induced anhysteretic moment.

As applied by Irving to five Cainozoic basalt samples of known orientation, the method seemed to work. And in a similar test, Brooke *et al.* (*Can. J. Earth Sci.*, **7**, 1515; 1970) obtained a success rate of 11 out of 12. But in a thorough study by Mohajer-Ashjai (*Can. J. Earth Sci.*, **12**, 62; 1975) on 20 lavas from Oregon and Mull, agreement between known and predicted polarities has been obtained in only 10 cases. Not surprisingly, Mohajer-Ashjai concludes that the Irving technique is not reliable enough. Although it is not completely worthless (since the probability of getting a 50% agreement purely by chance is less than 0.054) it seems that unoriented dredge samples will be of little use as a source of magnetic information about the oceanic crust.

closely related to the concentration of sheets, being less than 10° where sheets are scarce or absent but increasing to 35°–50° where they are most abundant. Lastly, and significantly, the direction in which the sheets dip is downwards towards the spreading axis—the same direction as the less steeply dipping country rocks.

How did this remarkable swarm originate? The answer, Walker argues, is by a very simple mechanism involving density contrasts between the Icelandic crust and the uprising magma. As basaltic magma rises, its density changes because of the onset of vesiculation caused by the exsolution of dissolved

gases. Generally, this change (decrease) will take place at depths less than 1 km, but different batches of magma with different volatile or phenocryst contents and different chemical compositions will begin to decrease in density at slightly different levels within this range.

The presence of these variations raises the possibility that under certain circumstances different batches of magma may behave in quite different ways as they move up through the crust. For example, the variations could be critical if the density–depth profiles for the magma were close to that for the solid crust, which seems to be the case in the upper 4 km. Below a depth of about 4 km, the density of all the magma is undoubtedly lower than that of the crust, and so the magma will rise in that region. In the upper 4 km, however, where the magmatic and crustal density profiles are comparable, two possibilities arise. If the density of the magma never anywhere rises above that of the crust, the magma will continue its ascent until it reaches the surface and eruption results. On the other hand, if the magma density does rise above the crustal density at some point, the vertical progress of the magma will be there arrested.

Walker's proposal is that some of the magma does one thing and some does the other. In particular, the magma that reaches a level at which its density equals that of the surrounding crust then ceases to rise and, instead, moves away laterally along an equi-density surface as an intrusive sheet. An obvious difficulty with this view is that in practice the solidified intrusive sheets are not actually horizontal but are inclined away from the spreading centre with an inclination that decreases up the dip. However, there are several factors which could account for equi-density surfaces of variable slope, including a non-even distribution of dykes across the width of the active zone, temperature variations in the country rock and an infilling of the voids in the country rock by zeolites in certain areas. All three could contribute to density variations in the crust, although the dyke distribution is thought to be the most important. In addition, however, as the magma sheet rises up-slope its density will decrease, especially if vesicles begin to form, and so it may actually cross equi-density surfaces and move towards crustal rocks of lower density.

According to Walker, therefore, the existence of the swarm of inclined basic intrusive sheets may be explained in terms of processes at the spreading axis, albeit modified by the particular crustal environment through which the axis passes. But if that is the case, why are such sheets limited to this particular

part of Iceland? Perhaps they are not. In Walker's view, the sheets are only exposed in this part of Iceland because the erosion here has been particularly severe. He proposes that the sheet swarm actually "constitutes a widespread and integral part of the structure of Iceland and is not merely a local phenomenon". Elsewhere it is not seen because it lies below sea level.

He then goes on to identify this widespread swarm with crustal layer 3. Of the four main crustal layers identified seismically beneath Iceland, layers 0, 1 and 2 have been interpreted reasonably as basalt lavas and associated rocks. But the nature of layer 3, which has an average thickness of 6 km and begins at an average depth of 3.5–4.0 km, is still unknown. Certainly a layer 3 of basic intrusive material should have the right P wave velocity (6.35 km s^{-1}). The next step should therefore be to test Walker's hypothesis by measuring seismic velocities within the sheet swarm in south-east Iceland. It is unfortunate that all previous seismic profiles were run outside the swarm or in parts of it where the concentration of sheets is very low.

Group selection

from Robert M. May

MANY animals seem to exhibit altruistic behaviour, whereby individuals sacrifice their own interests for the good of the group. The 'warning cries' uttered by some birds are an example: the individual puts his own life at greater risk for the benefit of his fellows. Many other apparent examples are to be found in the patterns of behaviour with which animals regulate their population density.

But natural selection acts on individuals, not groups. It has been argued that 'group selection' (the evolution of traits which are advantageous at the group level, but disadvantageous at the individual level) cannot take place within the classical Darwinian framework.

In many cases, closer study reveals that seemingly altruistic behaviour directly benefits the individual as well as his group, thus removing any paradox. In other cases, group selection can be seen to derive from kin selection: the altruist's actions are of direct advantage to his relatives, and therefore his gene type is favoured by his action, even if his own life is not. As W. D. Hamilton has pointed out, social insects probably exhibit extreme examples of kin selection. In the Hymenoptera, females are more closely related to their sisters than to their daughters, and so from a genetic point of view they do better to care for a sister than

to devote an equal amount of care to their own offspring.

There remain, however, examples of apparent group selection which cannot be evaded by the above arguments. Maynard Smith, Levins, Boorman and Levitt and others have tried to construct mathematical models which exhibit the phenomenon. These models envisage a population distributed over many islands or other such environmental patches, with occasional migration between patches. They seek to show that when a 'selfish gene' appears it will (by classical individual selection) take over the local population, but that the consequent disadvantage to this group as a whole may cause its extinction before the selfish gene can spread to the other populations. All these models require the most delicate tuning if the desired effect is to be exhibited, and none of them constitutes a truly plausible explanation for group selection.

Recently, two different models have been proposed, both of which exhibit group selection in a robust fashion.

The first of these was suggested by May, and has independently been elaborated at monograph length by Gilpin (*Group Selection in Predator-Prey Communities*, Princeton University Press, 1975). These models take advantage of specifically nonlinear aspects of predator-prey or plant-herbivore interactions, which can produce stable oscillatory patterns in population density. Small advantages to the selfish individual can now be nonlinearly magnified into disastrously large amplitudes of population oscillation for his group. The result is that group selection can be rapid and powerful; although selfish genes continue to appear by mutation, the groups in which they appear are quickly extinguished before they spread.

A simpler and more compelling mechanism has been pointed out by Wilson (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 143–146; 1975). As in essentially all these models, first suppose the total population is constituted of several isolated groups. Periodically these populations are mixed together and then re-dispersed. (One may think of the annual episodes of winged dispersal among so many insects.) The population contains two genotypes: altruists, *a*, and selfish non-altruists, *b*. Wilson notes that it can be that in each group the altruists suffer higher mortality than the non-altruists, but that nevertheless the overall proportion of altruists in the population can increase from generation to generation.

This statistical paradox is familiar in other areas, for example medical biometrics, and is best illustrated with a numerical example. To fix ideas, let *a* be birds which give warning cries,

thereby decreasing the average mortality in their group at the expense of increasing their own mortality rate relative to the selfish *b*. Suppose we have two patches: the first containing 25*a* and 75*b*, the second 75*a* and 25*b*. Initially *a* therefore constitutes 50% of the population. The birds in the first patch suffer higher mortality by virtue of the smaller complement of altruists; just before the next dispersal phase there are, say, 30 individuals, 6*a* and 24*b*. The second group does better, coming down to, say, 70 individuals, 50*a* and 20*b*. Note that in both groups the fraction of altruists, *a*, has decreased (from 25% to 20% in the first, and from 75% to 71% in the second), but that the altruistic genotype has increased in the population as a whole (from 50% to 56%).

Wilson's model has the quality of a *posteriori* obviousness which characterises so much of the best in science. It illustrates group selection, pure and simple.

Emplacement of intrusions

from G. M. Brown

THE geological evolution of the British Isles is a long and complex story but one period is particularly well understood, due to a prodigious amount of scientific investigation since the 1920s. Relatively recently in geological time, about 60 million years ago in the early Tertiary, active volcanoes erupted in what is now north-west Britain. Vast outpourings of basaltic lava were succeeded by the emplacement of a very complex array of igneous bodies in the form of dykes, cone sheets, ring dykes and other intrusive masses. The rock types are variable but include, predominantly, the products of medium to slow crystallisation of basaltic and granitic magmas. The intrusive complexes are believed to represent generally the eroded cores of central-type volcanoes that developed, as in Iceland, on and within the plateaux of the earlier flood lavas. Remnants of the flood lavas are preserved particularly well in Antrim but also in the Scottish Inner Hebrides. The later, central volcanic complexes constitute the bulk of many of the Hebridean islands such as Arran, Rhum, Mull and Skye, as well as Ardnamurchan (mainland Scotland) and the Mourne and Carlingford mountain regions in northern Ireland.

The volcanic regions have been mapped in ever-increasing detail, including geophysical measurement, by British geologists; and a host of laboratory studies has been conducted on the phase petrology, mineralogy and

geochemistry aimed at understanding the genesis of the rock suites. Yet the mechanism of emplacement of the intrusive complexes has remained a major problem. Walker (*J. Geol. Soc. Lond.*, **131**, 121; 1975) has now provided a first, really new and comprehensive explanation since the earlier hypotheses of Bailey, Richey, Harker and associates (chiefly 1904–1936). Those hypotheses were based chiefly on the concept that some intrusions were facilitated by pressures exerted upwards by underlying magma bodies while others were formed during the release of such pressures and the downward collapse of crustal blocks. The mechanisms were quantified by reference to Anderson's classic work (1936) on the dynamics of formation of cone-sheets (upward pressure), ring dykes and cauldron subsidences (downward collapse). Since then, refinement of Anderson's hypothesis rather than an alternative hypothesis has been the characteristic of modern, sparse work on the problem.

Walker's paper, in his own words, "... presents no new geological facts but sifts some of the great volume of existing data ...". The data are drawn from the meticulous observations and superb geological maps produced by the earlier Geological Survey staff referred to. From that, and his own observations in Iceland, he proposes radically that the main control of emplacement mechanics was the uprise of granitic diapirs in the region, early in the history of each Tertiary intrusive centre. The Goatfell granite body in northern Arran is quoted as a good example of such a diapir, but other examples are described. Granitic liquid would have a low magma density of about 2.3, and an analysis is made of the relationships between hydrostatic (magma) pressures and lithostatic (load) pressures in the vicinity of such diapirs. The construction of isopiestic (equal pressure) surfaces, after the manner of Bradley (1965), results in models that are convincing when related to the forms of intrusions in the volcanic centres, and to the early-published evidence for early doming and annular folding at most centres.

Basaltic magmas, associated with the main volcanic outpourings would, according to Walker, generate granitic (acid) magmas below the surface to the point at which a large body of granitic magma develops, rises as a diapir, and grows during ascent (100–1,000 Km³). Basaltic magma would be channelled into a magma trap at its base and develop into a cylindrical prism of basic intrusives as the diapir rises. The granitic body would release effusive magmas when it reached the surface, and thus diminish in size, while the basic rocks would rise in its wake.

View from Venus

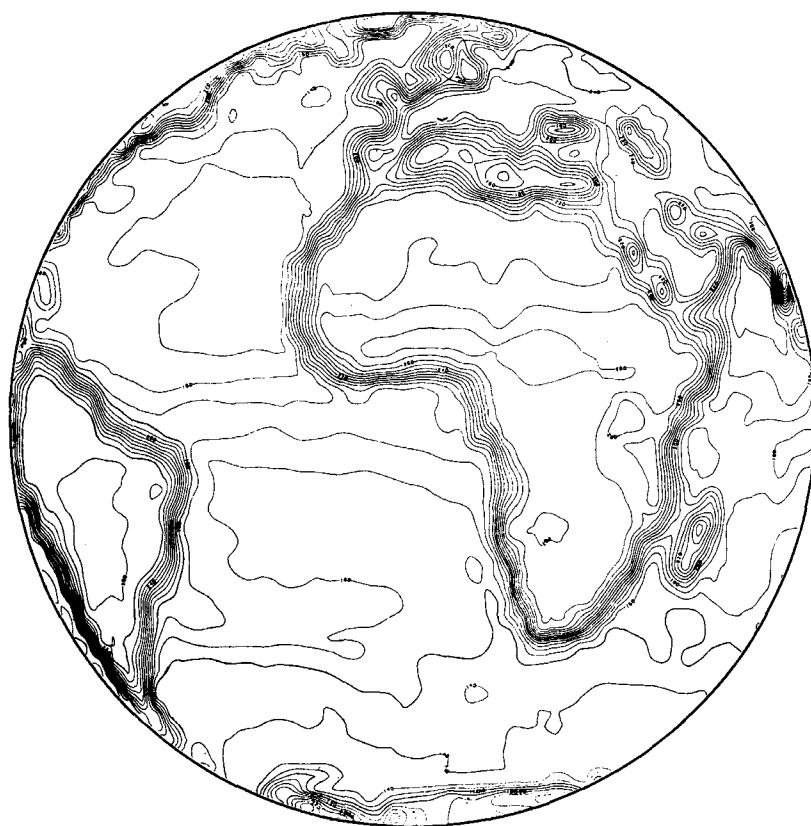


Image corresponding to 0 h. The contour interval is 10 K with every other contour labelled. From Webster, Chang, Darby and Finkelstein, *Icarus*, **24**, 144; 1975.

RADIO observations of the planets are all very well, but they would be better still if we had a benchmark against which to judge them. Webster *et al.* have now provided such a benchmark by using Nimbus 5 observations at 19.35 GHz to produce a "degraded and smoothed" simulation of what the Earth would look like to a radio astronomer on Venus armed with an instrument

with a resolving power of 1". The clear boundary between land and sea would, it seems, make it possible to determine very accurately the rate of rotation of the Earth, and also show up large permanent features (see figure). On this evidence and that obtained from radio studies of Mars and Venus, the Earth is undoubtedly the most interesting of the inner planets.

Other batches of magma would be diverted to zones outside the diapiric zones, where excess hydrostatic pressures existed (the model explains cone-sheet formation in this way). Late subsidence of the cylinder of basic intrusives below the diapir could provide for outward dipping structures such as ring-dyke and bell-jar (curved flange) intrusives.

The hypothesis is difficult to fault on the basis of a vast and otherwise embarrassing array of good quality, earlier field observational data. It is welcome because Anderson's model has long been found wanting, especially in regard to the role of his postulated ring-dyke mechanics. The need, now, is for a more rigorous consideration of

Walker's model, especially from a possible refinement of the density data and a more detailed analysis of pressure relations and diapiric movement applied to mobile granitic magmas. Certain assertions in the paper, peripheral to the main argument but nevertheless relevant, are not immediately acceptable. Walker refers to the Rhum ultrabasic rocks as representing ultrabasic magma (not necessarily so), and does not face up to the sequence of events in central Skye (granitic complexes later, rather than earlier than the Cuillins layered basic intrusives). He postulates a development of igneous layering parallel to the inclined floors of gabbro intrusions of "confluent cone-sheet type" whereas such layering, if con-

trolled by gravitational crystal settling, as he accepts it, is independent of a pre-existing floor slope. Some pertinent, recent publications on the Ardnamurchan cone sheets and the Skye hybrid (marscoite) bodies are not referred to. Early tidying-up is a natural requisite for an hypothesis that will need severe testing.

The mechanics of emplacement is a local problem, whereas the genesis of the British Tertiary magmas relates to universal problems. Work over the past decade or so has concentrated on the latter, and yet the origin of the granitic magmas remains in dispute. Most petrological, experimental and isotopic studies favour an origin by remelting of the crustal rocks (anatexis) rather than by crystal fractionation of the basaltic magmas. Walker's model leaves little doubt that only crustal remelting is acceptable, yet surprisingly he does not refer to that clue or to magma genesis in general. His granitic diapirs are shown as developing before the underlying cylinder of basic intrusives has crystallised, which would be impossible if they were late fractions of a solidifying basic intrusive, quite apart from the fact that only a very small volume of granite is produced by the latter process. Proponents of the fractionation hypothesis will not approve of Walker's granitic diapirs, which makes it all the more important that his hypothesis be explored to the full, and consideration be given to the several broader petrogenetic implications.

Silicon wafers without sawing

from Andrew Holmes-Siedle

ONE annoying deficiency in semiconductor device technology today is the limitation in size of the single-crystal ingots of semiconductors (such as silicon) which can be grown. Nearly all mass-produced ingots are grown by slow pulling from the melt (the Czochralski method). The best ingots that can be produced in this way are about two inches in diameter and six inches long. Thus, in silicon device factories, devices are formed on thin wafers cut from such ingots in a direction perpendicular to the long axis, like slices of sausage. Neither the size nor shape are conducive to good yield of devices in large production runs of transistors, integrated circuits or other 'small-signal devices'.

For the simpler solar cell diode, area is the dominant factor. Indeed, a square foot would be a good area for a single cell; such an area just cannot be obtained at the moment, even by the expensive method of cutting ingots along the long axis, as in sawing a

plank. Instead, an array of little cells has to be wired together, which greatly increases the cost and lowers the reliability of solar panels.

For these reasons, crystal growers have for a long time been exploring ways of making silicon crystals of larger area which might eliminate wasteful and arduous sawing and polishing. One technique which has recently raised hopes could be regarded as two-dimensional pulling of crystals. Crystallisation proceeds along a thin film of molten silicon lying in a mould. The method can be turned into a flow process if the film can be generated continuously. A ribbon, of theoretically unlimited length could then be produced. Tyco Laboratories, of Boston, have been exploring this process, termed edge-defined film-fed growth (EFG). As yet, few of the ribbons produced are single-crystal; most consist of large polycrystals which themselves contain imperfections in the form of dense tangles of dislocations. Nevertheless, there is often a dominance of one crystal orientation at the surface. It is thus possible that, under the right conditions, one might be able to promote totally single-crystal growth in a layer of silicon deposited on to the ribbon by one of the known epitaxial growth techniques.

Now one epitaxial growth technique is already widely used to improve the properties of integrated circuits formed on ingot-grown silicon wafers. This is the so-called chemical vapour deposition method. The layers grown are several micrometres thick and are of excellent single-crystal quality. The advantage of this deposition method is that only flowing gases are involved and the method, again a flow process, can probably be scaled up to cover large areas.

Thus, the process for making large, though imperfect silicon wafers in one step can possibly be married to this already widespread epitaxial growth technique to form a production method which yields large ribbons of crystalline silicon (it should be noted here that, for most active semiconductor devices, all of the electronic functions occur in a layer a few micrometres thick at one surface only).

Henry Kressel and co-workers at RCA Laboratories are the first to report this marriage. In *Applied Physics Letters* (25, 197; 1974) they note the happy result that, when the epitaxial growth from the vapour is carried out on ribbons of p-type silicon, made by Tyco Laboratories using the EFG technique, the defects in the original wafer are not strongly propagated into the newly forming crystalline lattice. This is shown clearly in X-ray topographs but more to the point is the finding that p-n diodes

with good minority-carrier lifetime, good breakdown voltages and low leakage currents can be made by putting down first p-type and then n-type layers on to this silicon ribbon. Although the ribbon used by the investigators were only 1.3 cm wide, there is no basic bar to scaling this up by an order of magnitude. Ribbon lengths of 2 metres, and widths of 2.5 cm have been reported unofficially.

For the integrated-circuit and transistor manufacturer, the availability of such ribbons could give the era of single-crystal devices a new lease of life, maintaining the price-competitiveness of this form against the rapidly developing and currently less expensive thin-film device fabrication methods.

For the solar array designers, the prospect of cheaper, more reliable solar panels is improved if the ribbon method succeeds. Indeed, it is probably no coincidence that the work at RCA took place under the supervision of Paul Rappaport, one of the pioneers of the silicon photovoltaic device and co-author of a recent survey for the National Academy of Sciences on the terrestrial use of solar converters. The latest news over the agency wires is that, despite some remaining problems with crystallinity, preparations are being made to make ribbon silicon on a factory scale.

Chromosomal proteins and control of transcription

from a Correspondent

The Florida Colloquium on Molecular Biology "Chromosomal Proteins and Their Role in the Regulation of Gene Expression" was held at the University of Florida in Gainesville on March 13 and 14.

ALTHOUGH there have been many reports correlating changes in non-histone chromatin proteins with alterations in gene expression, there has to date been little direct evidence linking the two processes. Encouraging however were reports from two laboratories of direct demonstrations that nonhistone chromatin proteins mediate control of transcription of the histone and globin genes. G. S. Stein (University of Florida, Gainesville) has synthesised a DNA complementary to histone mRNAs and used it to establish that the transcription of histone genes from chromatin is restricted to the S phase of the cell cycle. Chromatin re-

construction experiments showed that the nonhistone proteins of S phase chromatin are responsible for regulating the transcription of histone mRNA sequences. R. S. Gilmour and J. Paul (Beatson Institute for Cancer Research, Glasgow) have provided evidence using reconstruction experiments that in chromatin of mouse foetal liver cells and DMSO-stimulated Friend cells nonhistone proteins render globin genes transcribable. In both the histone and globin transcription studies the potential interference of endogenous RNA was ruled out. There has been some concern about both chromatin reconstitution and the use of bacterial polymerases for chromatin transcription, but the histone and globin systems at least seem amenable to these approaches.

Several laboratories are engaged in isolating and characterising defined nonhistone chromatin protein fractions and exploring their functions. H. Busch (Baylor College of Medicine, Houston) has been isolating chromatin and nucleolar proteins by differential salt extraction and fractionating them on a preparative scale by two-dimensional polyacrylamide gel electrophoresis. His group has purified and is at present sequencing a protein whose appearance is correlated with nucleolar activation.

Another technique which has been much used for fractionation of chromatin proteins is DNA affinity chromatography. V. Allfrey (Rockefeller University, New York) described new methods for the fractionation of nuclear nonhistone proteins which utilised DNA of various average *C_{ot}* values covalently attached to solid supports such as amino-ethyl sepharose, Sephadex G25 and Sephadex G200. This method yields reproducible fractions some of which interact preferentially with 'moderately reiterated' sequences while others bind to unique DNA sequences. Some of the DNA binding proteins exhibit a high and selective affinity for cyclic AMP or cyclic GMP. G. Patel (University of Georgia, Athens) described a class of proteins which do not exhibit species-specific binding but rather display a preference for AT-rich and single-stranded DNA. T. Y. Wang (State University of New York, Buffalo) reported the isolation of two nonhistone protein fractions from *Ehrlich ascites* tumour chromatin, one which activates and the other which suppresses transcription of DNA *in vitro*. The action of each of these protein fractions requires the homologous RNA polymerase II and homologous DNA. The inhibitory protein, which is electrophoretically homogeneous, blocks RNA synthesis at initiation prior to formation of the first phosphodiester bond.

There seemed to be a general consensus that DNA binding fractions are enriched in phosphoproteins. Though DNA affinity chromatography appears to be an effective method for fractionation of chromatin proteins, caution must be exercised in concluding that *in vitro* binding reflects biological activity.

T. C. Spelsberg (Mayo Clinic, Rochester) discussed the specificity of the binding of progesterone-receptor to hen oviduct nuclei and chromatin. Although multiple levels of binding were observed in all tissues examined, the highest affinity binding was only detected in chromatin from target tissues. The removal of one nonhistone protein fraction 'AP3' resulted in loss of high affinity binding.

For some time phosphorylation of chromatin proteins has been correlated with the activation of transcription in a variety of biological systems. Consistent with these observations, studies by R. Platz and L. S. Hnilica (University of Texas, Houston) indicated that during sea urchin development the rate and specificity of nonhistone protein phosphorylation reflects the process of differentiation.

L. J. Kleinsmith (University of Michigan, Ann Arbor) described the properties of two principal nuclear components of the chromatin protein phosphorylation mechanism. Twelve kinase activities were identified, several being cyclic AMP-dependent and others refractory to or even inhibited by cyclic nucleotides. Nuclear phosphatase activity was also described. Dr Kleinsmith, with G. Stein and J. Stein, has also found that enzymatic dephosphorylation of nonhistone chromatin proteins from S phase HeLa cells resulted in a reduction in chromatin template activity and in the transcription of histone mRNA sequences. The latter studies provide evidence for a functional relationship between phosphorylation of nonhistone chromatin proteins and RNA transcription.

● Histones were not entirely overlooked at the colloquium. R. Chalkley (University of Iowa) addressed the problem of deposition of preexisting and newly synthesised histones on replicating DNA. He showed that in contrast to DNA, which is replicated in a semiconservative fashion, histones are randomly distributed between the pre-existing and newly replicated DNA strands.

T. Langan (University of Colorado, Denver) presented evidence that there are two types of *F₁* histone phosphorylation, which can be distinguished on the basis of the specific amino acid residue phosphorylated and the requirement for cyclic AMP. Phosphorylation of serine 37 takes place in non-

proliferating cells following hormonal or cyclic AMP stimulation. Phosphorylation of amino acid residues other than serine 37 is strongly correlated with the rate of cell growth and is unaffected by cyclic nucleotides. Distinct enzymes which catalyse the two types of *F₁* phosphorylation reactions have been isolated. P. Byvoet (University of Florida, Gainesville) discussed the enigmatic phenomenon of histone methylation and its possible role in the maintenance of chromatin structure. He showed that agents which are capable of intercalating into the DNA double helix increased the extent of histone methylation.

● The last portion of the colloquium was concerned with the arrangement of proteins on DNA in chromatin. B. Sollner-Webb and G. Felsenfeld (NIH, Bethesda) have examined the structure of chromatin, both purified and within nuclei, by digestion with *staphylococcus* nuclease. The kinetics of nuclease digestion are consistent with the "beads on a string" arrangement of chromatin. Sollner-Webb and Felsenfeld also presented evidence which suggests that the organisation of proteins in the neighbourhood of globin genes differs between reticulocyte chromatin, which actively transcribes globin mRNA, and erythrocyte chromatin where globin transcription is largely repressed. In reticulocyte chromatin a distinct segment of the globin gene is not accessible to poly-D-lysine binding, presumably because it is covered by chromatin proteins. In erythrocyte chromatin the entire globin sequences seem to be present in both poly-D-lysine accessible and inaccessible regions. J. Gottesfeld and J. Bonner (California Institute of Technology, Pasadena) described a method for isolating a template-active chromatin fraction by digestion with DNase II. The DNA of this fraction contained a subset of the total genetic sequences which is partially tissue-specific. The template-active fraction has a decreased histone content, totally lacks histone I, and is enriched in non-histone protein. Limit digests of total chromatin with DNase II revealed nucleoprotein bodies equivalent to those described by Sollner-Webb and Felsenfeld. But limit digests of the template-active fraction did not yield these nucleohistone particles.

Correction

The letter in *Nature phys. Sci.* (230, 92; 1971) referred to in the News and Views article 'Polyacrylamide Gels' by Paul Calvert (254, 104; 1975) was by Richards and Temple, and not by Hersey and Rees as stated.

articles

Mantle deformation beneath the South African and Siberian platforms

G. D. Borley

Department of Geology, Imperial College, London, UK

Xenoliths of mantle lherzolite from kimberlite pipes in Africa and Siberian Yakutia have undergone deformation and thermal excitation of some of their constituent minerals. Their deformation states support the view that the mantle deforms systematically by creep processes resulting from shearing stress initiated, perhaps, during plate movement.

XENOLITHS thought to represent material from the deeper parts of the upper mantle are brought to the surface in kimberlite magmas. The most frequently occurring xenoliths are of lherzolite (olivine + ortho and clinopyroxene ± pyrope garnet); harzburgite (olivine + ortho-pyroxene ± pyrope garnet); eclogite (garnet + soda-alumina rich clinopyroxene); and pyroxenite. Less frequent, or rare, xenoliths are the kyanite and corundum eclogites, and grosspydites which contain a garnet rich in calcium, plus pyroxene and kyanite¹⁻⁶.

Lherzolite xenoliths from the kimberlite pipes in Africa provide evidence of deformation of the Earth's mantle⁷⁻¹⁵; they fall into broadly-defined textural categories.

Granular xenoliths of medium to coarse grain-size (1–5 mm), with weak microscopic fabrics, in which olivine and pyroxene show various strain effects, including undulose extinction, microfractures, kink and deformation bands and lamellae, slip-planes, and subgrain development, contain normal calcic-

diopsides that seemingly re-equilibrated at temperatures between 900 and 1,100 °C and at depths between 100 and 150 km (refs 7–10).

Xenoliths with variable microscopic fabric patterns containing porphyroclasts seem to have suffered increasingly intense shearing stresses through progressive deformation proceeding through a combination of creep (especially in olivine) and subgrain development. The diopsides in these lherzolites become increasingly subcalcic with deformation and seem to have re-equilibrated at temperatures between 1,100 °C and 1,400 °C at depths between 150 km and 190 km (refs 7–10). The most intensely deformed of these lherzolites grade into types with a distinctively striped foliation, and they contain sparsely distributed relic porphyroclasts of partly granulated pyroxene with porphyroclasts of undeformed pyrope garnet.

I have examined deformed lherzolites from African kimberlites contained in the Imperial College collection, similar lherzolites in the collection of A. Nicholas (Nantes University), and xenoliths from the Siberian Yakutia kimberlite pipes, in the collection of N. Sobolev.

These comparative studies of deformed lherzolites indicate that similar types of lherzolite from the deeper parts of the upper mantle in widely separated regions have had similar deformation histories. Compositional data on the minerals from these lherzolites also indicate that their thermal states may be comparable.

Table 1 Some analyses of coexisting Pyroxenes from lherzolites of Udachnaya Pipe, Yakutia* (see ref. 6)

	Yd. 5		Yd. 18		Yd. 161		Yd. 92		Yd. 111		Yd. 40	
	Diop.	Enst.	Diop.	Enst.	Diop.	Enst.	Diop.	Enst.	Diop.	Enst.	Diop.	Enst.
SiO ₂	55.5	58.5	54.9	58.6	55.7	58.0	54.9	58.4	54.5	59.0	54.4	58.8
TiO ₂	0.11	0.05	0.26	0.19	0.26	0.15	0.04	0.04	0.00	—	0.41	0.17
Al ₂ O ₃	1.11	0.65	1.47	0.54	1.23	0.50	0.82	0.46	1.01	0.33	1.57	0.61
Cr ₂ O ₃	1.10	0.29	2.00	0.43	1.98	0.50	1.08	0.36	1.08	0.25	0.88	0.27
FeO	3.07	4.95	3.32	5.33	3.25	5.50	2.96	4.93	1.67	4.64	3.12	5.53
MnO	0.11	—	—	0.13	0.05	0.13	—	0.08	0.00	0.13	0.07	0.11
MgO	19.5	35.8	18.4	35.6	19.4	34.7	19.5	35.4	17.7	36.3	18.1	35.5
CaO	17.5	1.03	16.9	0.86	16.8	0.80	19.2	0.85	21.6	0.39	17.3	0.67
Na ₂ O	0.98	0.17	1.66	0.24	1.5	0.29	0.91	0.12	0.84	0.04	1.68	0.19
K ₂ O	0.04	—	—	—	0.04	—	—	—	0.08	—	0.05	—
Total	99.02	101.44	98.91	101.92	100.21	100.60	99.31	100.64	98.51	101.08	97.58	101.85
Cations (on basis of 6 oxygen atoms)												
Si	2.009	1.979	1.994	1.978	1.994	1.985	1.992	1.991	1.996	1.996	2.001	1.985
Al (IV)	—	0.013	0.006	0.011	0.006	0.010	0.008	0.009	0.004	0.004	—	0.012
Ti	0.002	0.002	0.004	0.006	0.009	0.004	0.001	0.001	—	—	0.011	0.004
Al (VI)	0.048	0.013	0.059	0.011	0.046	0.010	0.027	0.009	0.040	0.009	0.071	0.012
Cr	0.030	0.008	0.057	0.011	0.056	0.014	0.031	0.010	0.031	0.006	0.027	0.007
Fe ²⁺	0.094	0.140	0.100	0.150	0.099	0.158	0.089	0.139	0.051	0.130	0.095	0.156
Mn	0.002	—	—	0.003	0.002	0.003	—	0.002	—	0.003	0.002	0.003
Mg	1.052	1.804	0.995	1.791	1.035	1.771	1.048	1.798	0.966	1.830	0.993	1.784
Ca	0.678	0.037	0.657	0.031	0.645	0.029	0.745	0.031	0.847	0.014	0.681	0.024
Na	0.070	0.011	0.118	0.016	0.103	0.019	0.065	0.008	0.062	0.002	0.119	0.013
Ca/(Ca + Mg)	39.2		39.8		38.4		41.6		46.7		40.7	

Petrography of deformed lherzolites

I will describe two lherzolites which are considered to be typical of the deformed varieties: one from the Jagersfontein (South Africa) kimberlite, and one from the Udachnaya kimberlite pipe in Siberian Yakutia.

In the lherzolite from Jagersfontein porphyroclasts of enstatite and diopside have been drawn out into lozenge and spindle shaped, partly granulated relicts and, together with undeformed porphyroclasts of pyrope garnet, they are set in a granoblastic-polygonal¹⁵⁻¹⁸ matrix of olivine grains 100 μm or larger in size. Small relict porphyroclasts of pyroxene merge outwards into irregularly shaped sub-grains with diffuse boundaries and finally (particularly in enstatite), into outer zones of polygonal grains many of which have triple-point junctions; these grains are of the order of 25 μm or less in size. Narrow zones of fine-grained enstatite run from the apices of the granulated relict porphyroclasts into the olivine matrix, which they cut sharply. These zones of crystals, which resemble stripes, give the most deformed lherzolites the foliation that typifies hand specimens; they seem to have been produced by a late, intensive, shearing during which stresses have been taken up by pyroxene rather than by the already deformed olivine. The lherzolite Ud.5 (Yd. 5 in Table 1) comes from the Udachnaya pipe which is located in the Daldyn-Alakit region of Siberian Yakutia north of the Arctic Circle and near the edge of the Siberian platform⁶. About 70 thin sections of some 40 lherzolite xenoliths from the Udachnaya pipe were examined. About 75% of these were deformed, the rest had coarse-grained, undeformed textures^{7-10,15}.

Ud.5 is typical of the deformed varieties and shows an early stage in the development of the striped foliation. It contains a few relict olivine porphyroclasts, together with elongate or lozenge-shaped porphyroclasts of partly granulated enstatite and diopside, and undeformed pyrope garnet. These porphyroclasts are set in an olivine matrix that has a granoblastic-polygonal texture and an average grain size between 70 and 100 μm .

The larger subgrains in the partly granulated enstatite have diffuse boundaries and merge into the relict porphyroclast; the small (25–30 μm), polygonal, grains are furthest away from the relict grain. Some zones of matrix olivine are separated from each other by the stripes, and some of the matrix olivine grains have flattened tops as though they were sheared off, or the grains changed shape by diffusion of material away from points of stress¹⁹. Both the Jagersfontein and the Udachnaya lherzolite xenoliths have textures transitional to the most intensely deformed lherzolites described from African kimberlite pipes^{11,12}.

Thermal history and origin

The pyroxenes in deformed or sheared lherzolites from African kimberlites have re-equilibrated at high temperatures and pressures⁷⁻¹⁰; the greater the degree of deformation the higher the temperature and pressure. Since many of the Udachnaya lherzolites show comparable degrees and types of deformation it is clearly important to determine whether their pyroxenes also re-equilibrated under high P/T conditions.

The temperature of re-equilibration of diopside that coexists with enstatite can be determined if the diopside contains low molecular proportions of ferrosilite and jadeite. In these circumstances re-equilibration temperatures can be obtained by applying the experimentally determined temperatures for compositions on the diopside–enstatite solvus at a pressure of 30 kbar to the $\text{Ca}/(\text{Ca} + \text{Mg})$ ratios of the natural minerals^{9,10,16}. Using this method for several diopsides from the Udachnaya lherzolites (analyses in ref. 6; selected examples in Table 1) gives re-equilibration temperatures between 1,130 and 1,250 $^{\circ}\text{C}$ for minerals from the deformed xenoliths, and a low temperature of 950 $^{\circ}\text{C}$ for diopside (Yd. 111 in Table 1) from a granular

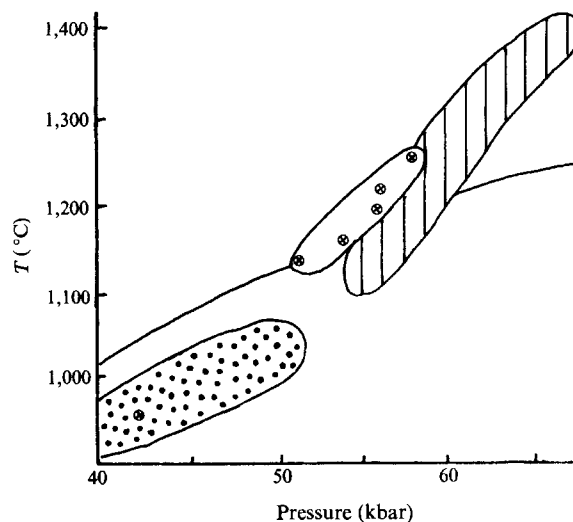
one. These temperatures compare with those obtained for diopsides from deformed and undeformed African lherzolite xenoliths⁷⁻¹⁰.

To estimate pressures of re-equilibration, and therefore depths of origin of the lherzolites, it is necessary to know the Al_2O_3 content of the enstatite that coexists with the diopside and pyrope garnet. Then, the experimental data on the solubility of Al_2O_3 in enstatite at different temperatures and pressures can be used¹⁷, after adjustment has been made to allow for Al_2O_3 combined with Na_2O in the jadeite molecule. Unfortunately data are not yet available on the pressures at which the solubility of Al_2O_3 in enstatite falls below 1%.

A few analyses of coexisting diopside–enstatite pairs from the Udachnaya garnet-bearing lherzolites are available (Table 1), and they show that a major problem in applying the experimental data on Al_2O_3 solubility in enstatite at different pressures is that the Udachnaya enstatites contain <1% Al_2O_3 . The amounts are reduced still further if allowance is made for Al_2O_3 combined with Na_2O in jadeite. But it seems reasonable to assume that the pressures at which the Udachnaya enstatites re-equilibrated were probably not less than those defined at various temperatures by the 1% Al_2O_3 isopleth in the experimental system¹⁷, and the latter has been used in conjunction with the re-equilibration temperatures from the coexisting diopsides to provide rough approximations of the pressures. This gives re-equilibration pressures of 50–59 kbar (or depths of origin between approximately 165 and 180 km) for the deformed lherzolites, and 42 kbar (depth of origin approximately 130 km) for the granular variety. Again, these figures compare with those for the African lherzolite xenoliths.

In Fig. 1 the data for the pyroxenes from Udachnaya lherzolites have been plotted in a way similar to that used for the African lherzolite xenoliths⁷⁻¹⁰. Accepting that compositional problems make the plots of the Udachnaya data slightly less accurate than those for the African material, two important features are still apparent. First, the data for the Udachnaya lherzolite xenoliths plot close to or in similar fields to the data for the African xenoliths. Second, the re-equilibration temperatures of diopsides from the deformed lherzolite xenoliths of both Udachnaya and African kimberlite pipes rise sharply with increasing pressure and both the trends are oblique to that for the shield geotherm. In effect, diopsides from both sets of deformed lherzolite xenoliths are thermally excited and their re-equilibration temperatures apparently lie along a geotherm referred to as 'perturbed'.

Fig. 1 Udachnaya pyroxene data. Stippled, diopsides from 'granular' lherzolites (Africa)⁹; hatched, diopsides from 'sheared' lherzolites (Africa)⁹; Udachnaya diopsides⁶; solid line, shield geotherm.



Further implications

Accepting that the observed deformation of the thermally excited lherzolites took place in the mantle it is possible, from the petrographic and chemical data, to make some general observations on the state of lherzolite assemblages below a depth of 150 km in the mantle below the African and Siberian platforms.

First, deformation of the lherzolites seems to be systematic, increases with depth of origin of the kimberlite magmas to at least 190 km and apparently proceeds through a combination of creep and subgrain formation. The deformation is accompanied by thermal excitation of the pyroxenes that coexist with pyrope garnet and olivine in these assemblages. Minor compositional differences in constituent minerals and therefore in the bulk lherzolite assemblages, for example like those that exist between the minerals of the Siberian Yakutia and African xenoliths⁶⁻¹⁰, do not significantly affect the general correlation between thermal and deformation states in lherzolites at depth (Fig. 1).

Second, the constituent minerals in the lherzolites deform in a systematic fashion^{11,12,15} and in a particular order. Olivine deforms more easily, and at an earlier stage, than enstatite, which seems to deform a little more readily than diopside. Pyrope garnet usually remains undeformed to depths of at least 190 km, though slight granulation of the mineral has been observed in one or two highly deformed lherzolites.

Third, there seem to be limits to the grain sizes attained by individual minerals during deformation. For example in early stages of deformation of the lherzolites, matrix olivine grains may be of the order of 300 μm or a little less¹¹, but in later stages, when enstatite and diopside have commenced granulation, matrix olivine grains may be of the order of 50–100 μm . Olivine grains smaller than 40 μm are infrequent in xenoliths I have examined. Enstatite forms small (25–35 μm) grains, during early stages of its deformation, but in the most deformed lherzolites enstatite grains as small as 10 μm have been observed^{11,15}. Enstatite, or diopside, grains smaller than this do not seem to have been described.

Fourth, there is no evidence of lherzolites in which diopside has re-equilibrated at temperatures higher than 1,450 °C (refs 9 and 10 and Fig. 1), although this may in part be a function of unavailability of material from depths much greater than 200 km. I note, however, that the T/P gradient for deformed lherzolites in Fig. 1 is much steeper than that of the shield geotherm, and it may be that further stress at depths >200 km could increase temperatures to the point where small volumes of partial melt were produced in the lherzolites, rather than further deformation and mineral re-equilibration.

A major problem in geology at the moment is to account

for the correlation between deformation states of lherzolite xenoliths from depths 150–190 km and the fact that P/T re-equilibration data for their pyroxenes plot along a 'perturbed' geotherm (Fig. 1). It has been suggested that this perturbation of the geotherm took place during plate movement when Gondwanaland fragmented during the Mesozoic, the resulting shearing stresses causing deformation and frictional heating in the lherzolite assemblages at depth⁹. A difficulty in totally accepting this view at the moment is that xenolith-bearing kimberlites from Africa and Siberia range in age from pre-Cambrian to post-Cretaceous and we do not know if deformation is restricted to lherzolite xenoliths that give Mesozoic dates. The age of the Udachnaya kimberlite pipe in Yakutia is unfortunately uncertain with K/A determinations on phlogopite and olivine giving dates ranging from upper Palaeozoic to late Jurassic respectively²¹. But it is clear that only a general, and major, event (or series of such events) could have produced comparable textures and thermal states in lherzolites from widely separated regions in the Earth's mantle. It may be, as some geologists now believe, that plate movement has been operative since the Precambrian and that deformed lherzolites provide a record of the way the mantle has reacted to stress over a long period of time.

I thank the Royal Society for a travel grant, the Royal Society and the Academy of Sciences of the USSR for arranging a visit to Siberia and Professor V. Sobolev and Dr N. Sobolev for help.

Received December 19, 1974; revised January 23, 1975.

- 1 Bobrieich, A. P., et al., *Petrography and Mineralogy of Kimberlite Pipes of Yakutia* (Nedra, Moscow, 1964). (In Russian.)
- 2 Sobolev, N. V., *Dokl. Acad. Sci. U.S.S.R.*, **157**, No. 6 (1964).
- 3 Sobolev, N. V., and Kuznetsova, I. K., *Dokl. Acad. Sci. U.S.S.R.*, **163**, No. 2 (1965).
- 4 Sobolev, N. V., *Proc. XXIII Int. Geol. Congress.*, **1**, 155–163 (1968).
- 5 Sobolev, N. V., *Proc. XXIV Int. Geol. Congress.*, Section 2, 297–302 (1972).
- 6 Sobolev, N. V., *The deep-seated inclusions in Kimberlites and the Problem of the Upper Mantle Composition* (Nauka, Novosibirsk, 1974). (In Russian.)
- 7 Boyd, F. R., and Nixon, P., *Ann. Rep. Geophys. Lab. Washington*, **71**, 362–373 (1972).
- 8 Boyd, F. R., and Nixon, P., *Ann. Rep. Geophys. Lab. Washington*, **72**, 431–446 (1973).
- 9 Boyd, F. R., *Geochim. cosmochim. Acta*, **37**, 2533–2547 (1973).
- 10 Nixon, P., and Boyd, F. R., in *Lesotho Kimberlites* (Lesotho Nat. Development Corp., Lesotho, 1973).
- 11 Boullier, A.-M., and Nicholas, A., in *Lesotho Kimberlites*, 57–66 (Lesotho Nat. Development Corp., Lesotho, 1973).
- 12 Cox, K. G., Gurney, J. J., and Harte, B., in *Lesotho Kimberlites*, 76–100 (Lesotho Nat. Development Corp., Lesotho, 1973).
- 13 Gueguen, Y., and Boullier, A.-M., in *Chemistry & Physics of Rocks & Minerals* (edit. by Strens, R. G.), (Wiley, in the press).
- 14 Borley, G. D., and Suddaby, P., *Min. Mag.* (in the press).
- 15 Borley, G. D., in *Chemistry & Physics of Rocks & Minerals* (edit. by Strens, R. G.), (Wiley, in the press).
- 16 Davis, B. T. C., and Boyd, F. R., *J. Geophys. Res.*, **71**, 3567–3576 (1966).
- 17 MacGregor, I. D., *Am. Mineral.*, **59**, 110–119 (1974).
- 18 Spry, A., *Metamorphic Textures* (Pergamon, 1969).
- 19 Stocker, R. L., and Ashby, M. F., *Revs Geophys. Space Phys.*, **11**, 391–426 (1973).
- 20 *Lesotho Kimberlites* (edit. by Nixon, P.), (Lesotho Nat. Development Corp., Lesotho, 1973).
- 21 Sarsadskich, N. N., Blagulkina, V. A., and Silin, YU. I., *Dokl. Acad. Sci. U.S.S.R.*, **168**, No. 2 (1966).

The chemistry of sea salt aerosol and its measurement

M. P. Paterson & R. S. Scorer

Air Pollution Research Group, Mathematics Department, Imperial College, London SW7, UK

The chemistry of precipitation (rain and snow) has been extensively monitored since the 1950s, with the analysis performed for nine ions in solution. It has been suggested that this monitoring should be extended to include the analysis for heavy metals. The examination of results which we report here indicate that routine measurement of one of the most common ions, sodium, has been of too low a quality to prove the most significant current theory about its behaviour in the atmosphere.

Of the nine ions which are measured¹— SO_4^{2-} , Cl^- , NO_3^- , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+} and H^+ —only sodium and chloride are closely related in atmospheric occurrence in that the major source of both is the sea. They occur in seawater in a Cl–Na ratio of 1.80 by weight, which is signified by R_s in this paper. If there were no other sources, and no separation of the two ions in sea salt aerosol, their weight ratio in samples of both aerosol and precipitation would always be R_s , whatever the degree of dilution. Other land sources of chloride^{2,3} and sodium⁴ have been suggested but not proven to be of any significance in precipitation chemistry. The most substantial and widely

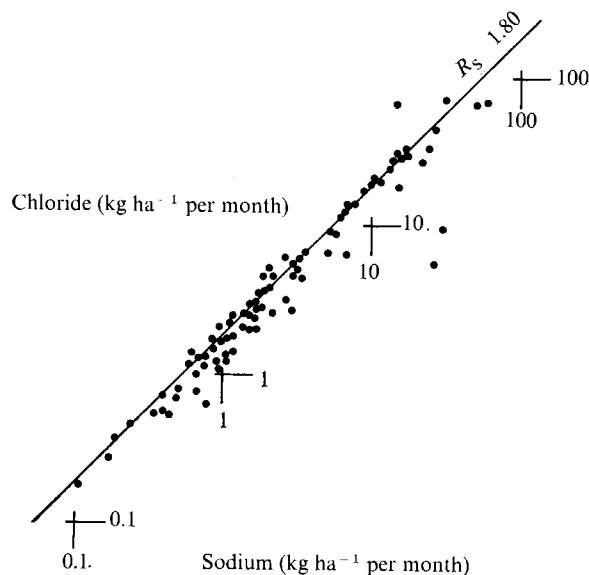


Fig. 1 Monthly amounts of chloride and sodium in precipitation at three coastal stations of the European Air Chemistry Network in southern Sweden and Norway, 23, 38 and 53, for the period 1960–62. The observed values of the Cl–Na weight ratio fall close to the ratio in seawater, 1.80, which is signified by R_s . Deviations from R_s are distributed similarly among months with large and small amounts of salt in precipitation.

accepted, if controversial, theory about sea salt in the atmosphere is the Cauer hypothesis⁶ which states that the sea salt chloride and sodium are separated by chemical or photochemical action in the air and that, from pure sea salt origins, the ratio of chloride to sodium in precipitation, signified by R , can range below and above R_s . Early measurements in Sweden⁶ seemed to bear out this theory but a re-examination of the data has suggested that the principal support for the theory, the large deficit of chloride in precipitation near the coast, resulted from incorrect chemical analysis⁷.

Discrepancies in monitoring data

An examination of monitoring data has shown discrepancies across national boundaries in Europe, similar to discrepancies in the calcium–magnesium ratio which have been reported elsewhere⁸. In the UK there is a discrepancy between results obtained by different laboratories. The dominance of sea salt as a source allows us to identify one laboratory as being most probably correct. A characteristic of the results from the other laboratory is found in data from monitoring networks in other parts of the world, suggesting that their results should be critically re-examined. Figure 1 shows the monthly amounts of sodium and chloride at several coastal stations in Norway and Sweden over a period of 3 yr. Deviations from R_s are equally distributed among months where the amounts of salt were large and small. In considering the distribution of the deviations from R_s we will disregard the absolute amounts of salt and treat only the value of R . We will consider the distribution in time of deviations and determine whether the observed distribution could arise from geochemical phenomena or from laboratory factors.

Where deviations from R_s result from phenomena which modify the sea salt while it is suspended as an aerosol, the same phenomena should affect samples at adjacent stations in the same month. A phenomenon which causes a high value of R at European Air Chemistry Network (EACN) stations 23, 38 and 53 in southern Norway and Sweden should also produce high values at stations 69, 70 and 71 in Denmark. Whether it would also affect station 54 in northern Norway is a matter for conjecture. A laboratory cause for a set of high values could be

either the dispatch of a contaminated set of bottles to the stations or a deviation in the analysis for either sodium or chloride when a month's samples have been returned. If it is a rare event it is unlikely to coincide with a similar event at another laboratory. We have selected the period 1959–63 for analysis because, for the first half of that time the Danish samples were analysed in Copenhagen with samples from 12 other Danish stations. The Norwegian and Swedish samples were analysed in Stockholm. The Danish analysis was discontinued in May 1961. After August 1961 the samples from Danish stations 69, 70 and 71 were analysed in Stockholm together with the samples from about 30 Norwegian and Swedish stations. We can thus compare, for the same set of stations, the degree of coincidence of high or low values of R when analysed at either one or two laboratories.

Table 1 shows the occurrence of values of R less than 1.50 and greater than 2.16 during the 60-month period. Samples were collected at each of the seven stations in 44 months, during which time the Danish samples were analysed in Copenhagen for the first 21 months. The results are expressed in the contingency tables (Table 2) which give the number of occasions on which none, one, two, three or four of the four Swedish and Norwegian stations experienced low (or high) values of R tabulated against the simultaneous occurrence of

Table 1 Cl–Na ratios in precipitation

	Norway and Sweden				Denmark		
	23	38	53	54	69	70	71
1959	•	•	•	•	•	•	•
	•	•	+	•	•	•	•
	•	•	+	+	•	+	–
	•	–	•	•	•	–	•
	•	•	•	–	•	•	•
	–	•	•	–	•	•	•
	–	•	–	•	•	–	•
	•	•	•	+	•	•	•
	•	•	•	–	–	–	–
	•	•	+	–	+	•	•
1960	•	•	•	•	•	•	•
	•	•	•	•	•	–	–
	–	–	•	–	•	•	–
	•	•	•	–	–	•	•
	+	•	•	•	•	–	–
	•	–	–	–	•	+	+
	•	•	•	•	–	–	–
	•	•	•	–	•	•	+
1961	•	•	•	•	•	+	+
	•	•	•	•	•	–	–
	•	•	•	•	•	•	–
	–	–	•	•	•	•	•
	+	+	•	+	+	•	+
	+	•	–	•	+	•	–
	–	•	–	–	•	–	–
1962	–	–	–	•	–	–	–
	•	•	–	•	•	•	•
	•	•	•	•	•	•	•
	•	–	–	•	–	–	•
	–	–	–	–	–	–	•
	+	•	+	+	+	•	•
	•	•	•	•	•	•	•
	•	–	•	–	•	•	•
	•	•	•	•	•	•	•
1963	–	–	–	–	•	•	–
	•	•	•	•	•	•	•
	•	–	+	–	•	•	•
	•	–	•	•	•	•	–
	•	–	•	•	•	•	•
	•	–	•	•	•	•	•
	•	–	–	•	•	•	•
	•	•	+	•	•	•	•
	+	+	+	•	+	+	+
	+	+	+	•	•	+	+

–, Indicates Cl–Na less than 1.50; •, indicates Cl–Na between 1.50 and 2.16; +, indicates Cl–Na greater than 2.16.

Table 2 Contingency tables

(a) (i) Low values ($R < 1.50$) (ii) High values ($R > 2.16$)

		3 Danish							3 Danish				
		0	1	2	3			0	1	2	3		
4	0	6	1	3	1	11	0	13	1	2		16	
Norwegian	1	3	2		1	6	1	3	1			4	
and	2		1			1	2		1			1	
Swedish	3	2	1			3	3						
	4					0	4						
		11	5	3	2	21			16	3	2	21	

(b) (i) Low values ($R < 1.50$) (ii) High values ($R > 2.16$)

		3 Danish							3 Danish				
		0	1	2	3				0	1	2	3	
4	0	9	1			10	0	16				16	
Norwegian	1	2	2			4	1	2	1			3	
and	2	4		1		5	2					0	
Swedish	3			1	1	2	3		1	2	1	4	
	4		1	1		2	4					0	
		15	4	3	1	23			18	2	2	1	23

low (or high) values at none, one, two or three Danish stations. Table 2a covers the 21 months during which two laboratories operated. Table 2b covers the period when all samples were analysed in Stockholm.

Table 2 shows that the two laboratories produce low (and high) values of R at different times. In the first period, when the analysis was carried out in separate laboratories, on none of the three occasions on which three or more of the Stockholm results fell below 1.50 did more than one Danish result fall below 1.50. From Table 2a (i), we see that of the five occasions on which two or three of the Danish stations recorded low results, on none of these did more than one Swedish or Norwegian station record a low result. Similarly with the occurrence of high values, in Table 2a (ii), although the situation is less clear in this as only thirteen high values were recorded at the seven stations in 21 months.

In the second period, when all samples were analysed in Stockholm, a clear association appeared between high (or low) values in Denmark and high (or low) values in Sweden and Norway. Table 2b (i) shows that on four occasions low values were recorded for two or more stations in each section of the network, and b (ii) shows that on three occasions high values were recorded at at least two stations in each section of the network.

What does this tell us about the records of the Network? There is a factor, unrelated to the chemistry of sea salt aerosol or precipitation, which leads occasionally to the finding of Cl-Na ratios in precipitation which deviate significantly from the sea salt ratio. If we can take the simultaneous presence of three or four deviant values out of a possible four as evidence of its occurrence then the results of most of the Norwegian and Swedish stations are affected by it one month in four. In assessing the significance of the factor to the records of the EACN we must consider not the number of occasions on which it occurs but the number of observations which are changed by the factor. The occurrence of three low values of R

in Denmark and three in Norway and Sweden in one month, counted as a single occurrence of the factor in the contingency tables above, will affect the records as much as single occurrences of low values of R on six different months. Seen in this light Table 2b (i) and (ii) indicates the following score for seven stations during 23 months (Table 3).

If this interpretation is accepted then it seems that at least 23% of the coastal precipitation sample analyses in Stockholm deviate significantly from R_s because of the factor. Not more than 17% deviate from R_s because of other causes. Thus, there is no strong evidence for a deviation from the sea salt ratio as a result of natural causes such as have been proposed by Rossby and Egnér⁶ or by Eriksson⁹. If any such deviation does occur at coastal stations it is so subtle as to be masked by an artefact of the network which we believe to be a laboratory factor.

In the UK, where precipitation sampling and chemical analysis has been performed since 1957 by the Meteorological Office in the traditional EACN way, a second programme has been commenced by the Atomic Energy Research Establishment (AERE) using neutron activation, X-ray fluorescence and other techniques which differ from those used by the EACN. The AERE results which we quote here have been taken from a report which was published in 1972¹⁰. The AERE monthly results for Wraymires, in Cumbria, in 1971 are plotted on Fig. 2a with Cl and Na on logarithmic scales. In all months R falls close to R_s . When the excess Cl, or Na, is estimated for each month the total of each for the year is a 3% excess of Cl and a 5% excess of Na, which are barely greater than the measurement errors. At the EACN station at Eskdalemuir, for which the monthly amounts of Cl and Na are shown in Figure 2b, there were no months with excess Cl. The excess Na amounted to 97% of the Na from a presumed sea salt source. It would not be unreasonable to assume that at Eskdalemuir, 110 km north of Wraymires, there is a land source which, throughout the year, contributes as much sodium to the precipitation as does the sea. Such an assumption is not borne out by an examination of the Lerwick results for the same period.

At Lerwick, which is a maritime site receiving ten times as much sea salt as Eskdalemuir, there is also a great excess of sodium, as seen in Fig. 2b. Could this come from a local land source? The excess amounts to 25% of the sea salt sodium over the year and in total quantity is 2.5 times the excess at Eskdalemuir. It would require a very strong land source of sodium in the Shetlands for such an observation to be real. But if there were a Shetlands source it would have to operate in sympathy with that near Eskdalemuir because the two stations experience high, and low, values of R in the same months. Figure 2b shows that in 1971 the low values of R occurred at both stations between April and September. It is most unlikely that so many common factors relate the chemistry of precipitation at Lerwick and at Eskdalemuir, which are 550 km apart and very differently exposed to sea and land sources, while Eskdalemuir and Wraymires, which are 110 km apart and similarly exposed, have little in common if the data is all to be believed. The significant factor relating Lerwick and Eskdalemuir is that samples from both are analysed in the one laboratory. Yet it is the Wraymires results, from a different laboratory using quite different analytical methods, which conform to the most reasonable expectation about the

Table 3

	Number	%
Total number of observations	161	
Observations of R between 1.50 and 2.16	96	60
Low values attributable to the factor	21	13
Low values not necessarily attributable	20	12
High values attributable to the factor	16	10
High values not necessarily attributable	8	5

chemistry of precipitation. This is that the predominant source of both chloride and sodium is the sea, and that these two ions remain associated during their transport on the wind in just the way they are associated in seawater. It seems that the system which produces the EACN results should be examined to discover the cause of the incorrect sodium or chloride analyses. J. L. Brownscombe and M. S. Porter (Meteorological Office, private communication) report that the phenomenon to which B. C. Oddie referred has recurred in some but not all of the later years and that it does not occur in all of the three rain ranges now operating at Lerwick.

The UK is just one of a number of countries which are participating in the EACN. Although there are minor local variations, all laboratories are using a similar set of analytical methods. Only in the UK can we be reasonably certain about the deficiencies of the EACN precipitation analysis. We would like, though, to point out certain similarities in results from other countries which might indicate that similar deficiencies exist there.

In Fig. 3a the annual total of Cl and Na are plotted for a number of UK stations for the years 1960-62. The most maritime station, Lerwick, receives an apparently small fractional excess of sodium and the inland stations, including Eskdalemuir, receive larger fractional excesses. That excess was less in 1960-62 than in 1971, also shown in Fig. 3a, the year for which we have compared Eskdalemuir and Wraymires. But the previous section of this paper has demonstrated that the excess at Eskdalemuir is almost certainly illusory. On common-sense grounds, and the basis of the experience at Scandinavian coastal stations which were discussed at the beginning of this paper, there is even more reason to believe that the excess sodium at Lerwick is illusory. Thus, the fractional deviations from R_s , which are largest where the amount of salt is least, seem to be generated by deficiencies in the system. But the UK is not the only country to record such characteristics. In Fig. 3b are plotted for various countries the annual amounts of chloride and sodium for several stations in the years noted on the diagram. It is evident that in broad terms the results from each of these are similar to those for the UK. This is not indisputable proof that there are deficiencies in analysis in each of these countries. But before the

Fig. 2 For 1971, the monthly amounts of Cl and Na in precipitation recorded: a, by the AERE at Wraymires (●); b, by the Meteorological Office at Eskdalemuir (○, ●) and at Lerwick (△, ▲). In b the months between April and September are shown with solid symbols.

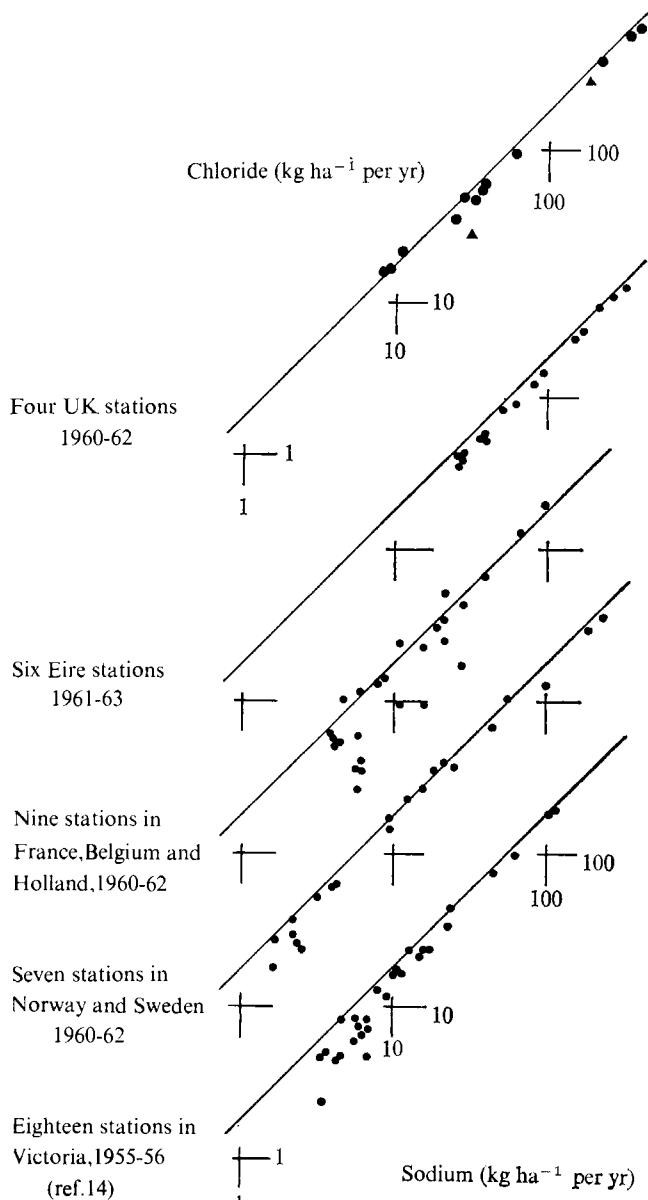
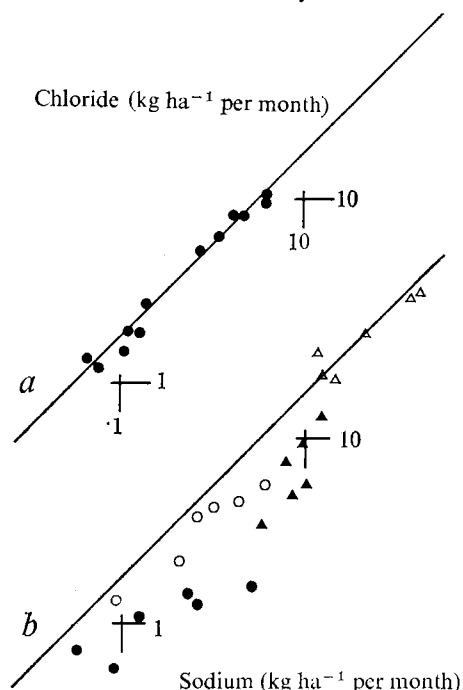


Fig. 3 Annual amounts of chloride and sodium in precipitation at a number of stations of the EACN in various countries, and in Victoria, Australia. In each country there is a tendency for excess sodium to occur, the fractional excess over sea salt being greater where salt amounts are least.

excess sodium is advanced as evidence of either land sources of sodium or chemical processes in the air transforming the sea salt aerosol, as for example that by Oddie¹¹, the possibility that it is an artefact of the system of chemical analysis should be considered. Before any further monitoring is done such artefacts should be eliminated.

There is an increasing awareness that data quality control programmes must be built into routine monitoring of the environment^{12,13}. The EACN experience with sodium and chloride analysis should alert us to one hazard of such programmes: if all laboratories cooperating in a programme of sample exchange are using the same analytical methods, or perhaps the same sample bottles, there is a possibility that all are recording the same bias, thereby allaying justified fears about the various possible forms of interference with results. While the internal consistency of data from coastal stations has given us a sufficient test when sea salt is plentiful, it was only the availability of a novel analysis which allowed us to determine that sea salt is the principal source of both sodium and chloride in precipitation at some inland stations.

We thank participants in the European Air Chemistry Network for making their results freely available. This work

has been supported by a research contract from Shell International Petroleum.

Received January 3, 1975.

¹ Egnér, H., Brodin, G., and Johansson, O., *Kungliga Lantbrukshögskolans annaler*, 22, 369 (1955).

² Hutton, J. T., *Arid Zone Research XI*, 285, UNESCO, Paris (1958).

³ Gorham, E., *Phil. Trans. R. Soc.*, B241, 147 (1958).

⁴ Junge, C. E., and Werby, R. T., *J. Meteorology*, 15, 417 (1958).

⁵ Cauer, H., *Der Balmologe*, 5, 409 (1938).

⁶ Rossby, C.-G., and Egnér, H., *Tellus*, 7, 118 (1955).

⁷ Paterson, M. P., *Tellus* (in the press).

⁸ Paterson, M. P., and Scorer, R. S., *Atmosph. Environ.*, 7, 1163 (1973).

⁹ Eriksson, E., *Tellus*, 12, 63 (1960).

¹⁰ Cawse, P. A., and Peirson, D. H., *AERE R 7134* (HMSO, London, 1972).

¹¹ Oddie, B. C. V., *Q. Jl R. met. Soc.*, 85, 163 (1959).

¹² Levadie, B., *WMO Report*, No. 368, 604 (1974).

¹³ Harley, J. H., *WMO Report*, No. 368, 629 (1974).

¹⁴ Hutton, J. T., and Leslie, T. I., *Aust. J. agric. Res.*, 9, 492 (1958).

Paracoccus denitrificans and the evolutionary origin of the mitochondrion

Philip John & F. R. Whatley

Botany School, South Parks Road, Oxford OX1 3RA, UK

It is demonstrated that Paracoccus denitrificans resembles a mitochondrion more closely than do other bacteria, in that it effectively assembles in a single organism those features of the mitochondrial respiratory chain and oxidative phosphorylation which are otherwise distributed at random among most other aerobic bacteria. A feasible evolutionary transition from the plasma membrane of an ancestral aerobic bacterium resembling P. denitrificans to the inner mitochondrial membrane is suggested.

ACCORDING to the endosymbiotic theory of the evolutionary origin of the eukaryotic cell, as developed by Margulis¹, the mitochondrion has evolved from a free-living prokaryote resembling a present-day aerobic bacterium, which had been taken up by a plastid-free, amoeboid cell, the protoeukaryote, that depended on fermentation for the production of ATP. In time the prokaryote suffered a progressive loss of autonomy; its proliferation kept pace with the cell division of its host; many of its biosynthetic capabilities were lost or taken over by its host; and its metabolism became integrated with that of its host.

The endosymbiotic theory implies that the outer mitochondrial membrane is derived from the protoeukaryote, and that the inner mitochondrial membrane and its invaginations (the cristae) are homologous with the plasma membrane of present-day bacteria. Here we describe the modifications of the bacterial plasma membrane which would be necessitated by the evolutionary transition from a free-living, aerobic bacterium resembling *Paracoccus denitrificans* (previously *Micrococcus denitrificans*²) to a mitochondrion. In this transition the ATPase and the constitutive components of the respiratory chain are retained; the adaptive components of the respiratory chain are lost; and the transport properties of the plasma membrane are altered so that, in its new role as the inner mitochondrial membrane, it is able to integrate the metabolism of the organelle with that of the surrounding cell. We chose *P. denitrificans* as a plausible ancestor because, when all possible biochemical parameters are compared, it resembles a mitochondrion much more closely than do other aerobic bacteria. This comparison is summarised below and will be presented in detail elsewhere³.

Comparison

Those mitochondrial features which *P. denitrificans* possesses, and which have a widespread (or perhaps universal) distribution among those bacteria which can make a respiratory chain, include nicotinamide dinucleotide trans-

hydrogenase^{4,5}, NADH and succinate dehydrogenases⁶⁻⁸, and the tricarboxylic acid cycle^{9,10}.

Those mitochondrial features which *P. denitrificans* possesses, but which have a limited distribution among other bacteria, include phosphatidyl choline as a major constituent of the membrane phospholipid fraction^{11,12}, straight-chain saturated and unsaturated fatty acids accounting for nearly all the membrane fatty acids^{11,13,14}, ubiquinone-10 as the sole functional quinone of the respiratory chain^{6,15,16}, two *b*-type and two *c*-type cytochromes as easily distinguishable components of the respiratory chain of aerobically grown cells¹⁶⁻¹⁹, cytochrome *aa₃* as the cytochrome oxidase^{6,16,20}, and a sensitivity to low concentrations of antimycin A and rotenone, both of which are inhibitors of mitochondrial electron transport^{16,21} (see Fig. 1). Furthermore, mitochondrial cytochrome *c* more closely resembles the soluble cytochrome *c* isolated from *P. denitrificans* than the *c*-type cytochromes isolated from other bacteria, in both physical and structural properties²²⁻²⁴. The soluble cytochrome *c* of *P. denitrificans* is unusual among bacterial *c*-type cytochromes in that it is to a large extent interchangeable with mitochondrial cytochrome *c* in its ability to react with mitochondrial cytochrome oxidase^{22,25-27} and with the two cytochrome oxidases of *P. denitrificans*, that is, the constitutive cytochrome *aa₃*^{27,28} and the inducible cytochrome *cd*, which functions *in vivo* as a nitrite reductase^{28,29}.

Furthermore, *P. denitrificans*, like *Hydrogenomonas eutropha*³¹ but unlike *Escherichia coli*³² and *Bacillus megaterium*³³, resembles a mitochondrion^{34,35} in the stoichiometry of oxidative phosphorylation as indicated by measuring H⁺/O ratios. Phosphorylating particles prepared from the plasma membrane of *P. denitrificans* show a mitochondrial type of respiratory control^{36,37} that is rarely observed in other phosphorylating bacterial preparations^{8,38}. As in mitochondria the respiratory rate is increased by the addition of ADP (respiratory control), or by the addition of carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) (an uncoupler of oxidative phosphorylation) and decreased by the addition of venturicidin which, like oligomycin, inhibits ATPase³⁹ (Fig. 2).

We should like to emphasise that although none of these mitochondrial features is unique to *P. denitrificans* no other species of bacterium possesses as many³. *Rhodopseudomonas spheroides*, however, when grown aerobically in the dark has a mitochondrial type of respiratory chain¹⁸, but the stoichiometry of oxidative phosphorylation and the presence of respiratory control are not known. Future research will probably reveal that *P. denitrificans* is not unique in the degree to which it resembles a mitochondrion, and it will then be viewed as a representative of a small group of aerobic bacteria (probably including *Rps. spheroides*) all having an obvious affinity with the mitochondrion.

In addition to the mitochondrial features found in *P.*

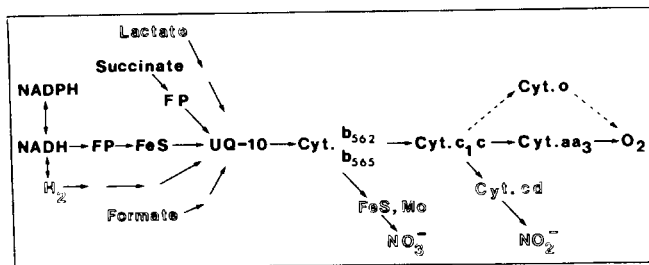


Fig. 1 The respiratory chain of mitochondrion and of *P. denitrificans*. Components of the mitochondrial respiratory chain, and of the constitutive respiratory chain of *P. denitrificans* are in heavy print; the additional adaptive components of the respiratory chain of *P. denitrificans* are in lighter print. Cytochrome *o* has been found in the mitochondria of only a few organisms⁶⁸, and its significance in the constitutive respiratory chain of *P. denitrificans* is not known. From ref. 3.

denitrificans described above, there seems to be no significant feature of the mitochondrial respiratory chain or ATPase which is present in another bacterium, but known to be absent from *P. denitrificans*.

Most of the differences between the respiratory chains of a mitochondrion and *P. denitrificans* can be accounted for by the presence in *P. denitrificans* of adaptive components which are absent from mitochondria. These adaptive components may be viewed as *ad hoc* additions to the constitutive portion of the respiratory chain, which is itself essentially similar to the mitochondrial respiratory chain (Fig. 1). The constitutive components of the respiratory chain of *P. denitrificans* are nicotinamide nucleotide transhydrogenase, NADH and succinate dehydrogenases, flavo-protein, iron-sulphur proteins, ubiquinone-10, cytochromes of the *b*-type and *c*-type, and cytochrome *aa3*. The adaptive components are hydrogenase, formate and lactate dehydrogenases, nitrate reductase, and nitrite reductase (cytochrome *cd*) (Fig. 1).

In their criticism of the endosymbiotic theory, Raff and Mahler⁴⁰ pointed out that, although aerobic bacteria possess cytochrome respiratory chains similar in function to mitochondrial cytochromes, "there are significant differences which suggest a considerable evolutionary divergence between mitochondrial and bacterial cytochromes". The differences cited are (1) the insensitivity of bacterial electron transport systems to some of the generally used inhibitors of mitochondrial electron transport; (2) the greater variety of bacterial cytochromes; (3) the poor cross reactivity of mitochondrial cytochrome *c* with most bacterial cytochrome oxidases, and vice versa; and (4) the dissimilarities in the primary sequence, redox potential and isoelectric point of bacterial and mitochondrial *c*-type cytochromes. We have noted above that while differences (1), (3) and (4) may apply to bacteria in general, these differences do not apply to *P. denitrificans* and *Rps. spheroides* since in these respects these bacteria resemble the mitochondrion while most other aerobic bacteria do not. Furthermore, it is shown below that the presence in *P. denitrificans* of "bacterial" cytochromes in addition to "mitochondrial" cytochromes raises no serious objection to an evolutionary transition from an aerobic bacterium resembling *P. denitrificans* to a mitochondrion, since these "bacterial" cytochromes are readily dispensed with under the appropriate environmental conditions.

Hypothetical evolutionary transition

A hypothetical transition from an aerobic bacterium resembling *P. denitrificans* to a mitochondrion, as envisaged by the endosymbiotic theory¹, would involve the loss of the adaptive components and the retention of the constitutive components of the respiratory chain of *P. denitrificans*

(Fig. 1). The synthesis of the adaptive components in *P. denitrificans* is readily suppressed in the presence of alternative electron donors or electron acceptors that are "more acceptable". Hydrogenase, for example, is present only when cells are grown in the presence of hydrogen and in the absence of organic compounds such as glucose⁴¹, and nitrate reductase is absent from cells grown aerobically in the presence of nitrate⁴². Similarly cells of *P. denitrificans* grown anaerobically in the presence of nitrate can use either nitrate or oxygen as terminal electron acceptors but, when both are supplied, use oxygen in preference to nitrate^{3,43}.

The postulated evolutionary transition does not require the adoption of new features by the constitutive respiratory chain of *P. denitrificans*, although modification of some components may occur. The limited extent of such changes is clear from X-ray structure analyses⁴⁴ of the readily solubilised *c*-type cytochromes of *P. denitrificans* and of the mitochondrion. The indications are that there is a very considerable similarity in the amino acid sequences of the two cytochromes⁴⁴. The exact extent of this similarity will be revealed by the detailed amino acid sequencing now being carried out (E. Margoliash, personal communication).

The similarity in the H^+/O ratios observed with *P. denitrificans* and with the mitochondrion imply that the respiratory chain is arranged in a similar way in the plasma membrane of *P. denitrificans* and in the inner mitochondrial membrane. The ATPases are also essentially similar in their mode of operation in *P. denitrificans* and in a mitochondrion⁴⁴, suggesting that the process of oxidative phosphorylation need not be altered during the evolution of the mitochondrion.

So far, the proposed evolutionary transition from an aerobic bacterium resembling *P. denitrificans* has not necessitated the acquisition by its plasma membrane of any entirely new features, nor has it involved a significant alteration in the mode of operation of those features already present. When the respective transport systems of *P. denitrificans* and a mitochondrion are compared, however, it becomes apparent that there are mitochondrial features not found in bacteria, such as *P. denitrificans*, as well as bacterial features not found in mitochondria. Furthermore, some carriers operate in a different way in bacteria and mitochondria.

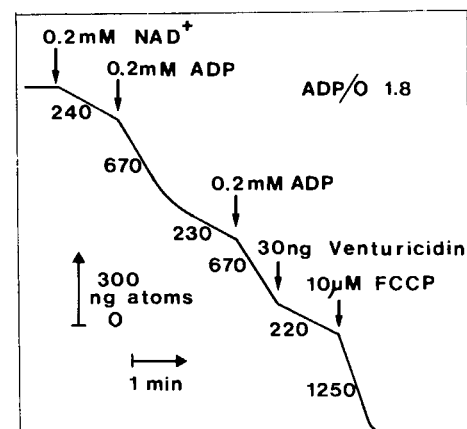


Fig. 2 Mitochondrial type of respiratory control in particles prepared from *P. denitrificans*. Membrane particles were prepared by a slight modification of the procedure of John and Hamilton³⁷. The reaction mixture contained, in a total volume of 3 ml: 30 μmol Tris-acetate (pH 7.3), 15 μmol magnesium acetate, 30 μl ethanol, 0.1 mg alcohol dehydrogenase (Sigma A7011), and 0.54 mg particle protein. Further additions were made as indicated. Oxygen uptake was measured in a Clark-type oxygen electrode at 30 °C. The ADP/O ratio was calculated as described for mitochondria by Chance and Williams³⁸. The numbers alongside the traces refer to the rates of oxygen uptake in ng atoms per min per mg protein.

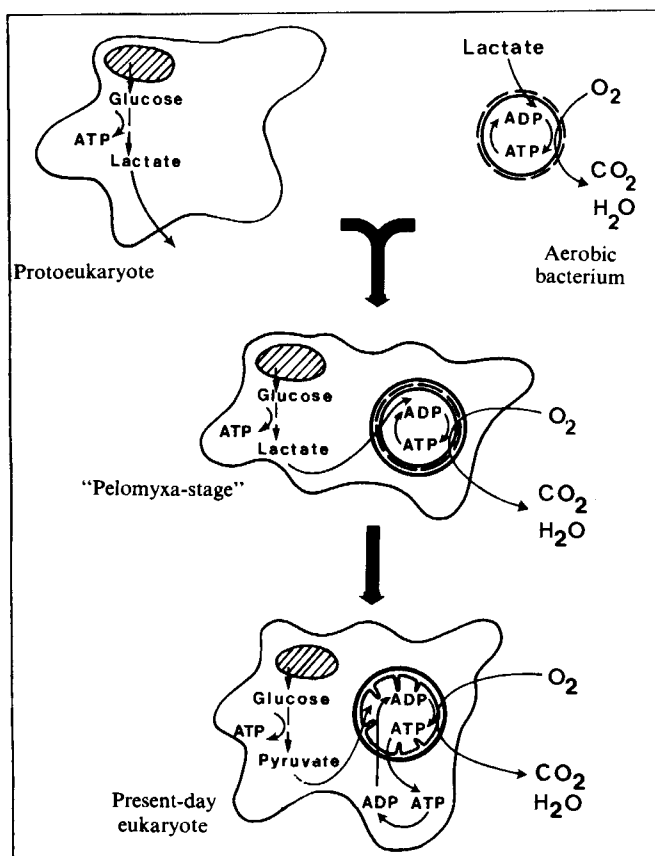


Fig. 3 Hypothetical evolutionary transition from a free-living aerobic bacterium to a mitochondrion. A fermenting protoeukaryote takes up a respiring bacterium to the advantage of both partners (the "Pelomyxa-stage", where lactate, the end product of fermentation, serves as the respiratory substrate for the bacterium). By the acquisition of an adenine nucleotide carrier the ATP synthesising potential of the bacterium is made available to the host cell (present-day eukaryote, containing mitochondria).

As with those features of the respiratory system, which are present in *P. denitrificans* but are absent from mitochondria, so also the transport systems which are uniquely bacterial can be related to the potentially more varied and unstable environment of bacteria compared with that of mitochondria. These bacterial transport systems, which would be lost in the transition to a mitochondrion, include carriers responsible for sugar transport, the periplasmic binding proteins and the extracellular iron chelators^{45,46}.

Both the plasma membrane of *P. denitrificans* (J. Burnell, P. J., and F. R. W., in preparation) and the inner mitochondrial membrane⁴⁷ contain a sulphhydryl-sensitive, phosphate carrier which mediates the uptake of P_i coupled to the simultaneous uptake of protons by an electroneutral process equivalent to the proton-symport of Mitchell⁴⁸. Thus this carrier, already present in the plasma membrane of *P. denitrificans*, would require no modification to operate in the mitochondrion.

On the other hand, the sulphate carriers in *P. denitrificans* and in a mitochondrion seem to operate by different mechanisms. In *P. denitrificans* sulphate is accumulated by an electroneutral proton-symport (J. Burnell, P. J., and F. R. W., in preparation) whereas, in mitochondria, sulphate permeates the inner mitochondrial membrane principally in exchange for succinate or malate⁴⁹. Similarly, while carboxylate carriers in a number of aerobic bacteria⁵⁰⁻⁵³ probably bring about the uptake of carboxylates coupled to the simultaneous uptake of protons⁴⁶, mitochondrial di- and tricarboxylate carriers act as exchange

diffusion carriers, mediating the electroneutral, equimolar P_i -dicarboxylate, dicarboxylate-tricarboxylate, and heterologous dicarboxylate-dicarboxylate exchanges⁴⁷. The carboxylate exchange carriers of the inner mitochondrial membrane clearly function to integrate the operation of the tricarboxylic acid cycle in the mitochondrial matrix with extramitochondrial metabolism⁵⁴. On the other hand, the carboxylate carriers of the bacterial membrane function simply in the accumulative uptake of carboxylates from the bacterial environment. Thus in an evolutionary transition from an aerobic bacterium resembling *P. denitrificans* to a mitochondrion, modification would be necessary to the mechanism of the sulphate and carboxylate carriers from a proton-symport (equivalent to an hydroxyl-exchange) to a sulphate-carboxylate, carboxylate-carboxylate, and carboxylate- P_i exchange.

The only new component necessary for an evolutionary transition from the plasma membrane of *P. denitrificans* to the inner mitochondrial membrane is the adenine nucleotide carrier⁵⁵, which seems to be absent from all bacteria and present in all mitochondria. This carrier makes ATP, synthesised on the matrix (inner) side of the mitochondrial membrane⁵⁶, available to the ATP-utilising sites in the rest of the cell.

If it be assumed that the protomitochondrion was tolerated by its host for a sufficient length of time for a stable symbiotic relationship to have developed before the acquisition of the adenine nucleotide carrier by the protomitochondrion, then it is necessary to consider what advantage this protomitochondrion may have conferred on its host for the symbiotic relationship to have survived the selective pressures to which it was exposed. We suggest that the protomitochondrion, by virtue of its tricarboxylic acid cycle and respiratory chain, was able to completely oxidise fermentation products such as lactic acid and ethanol, which may have accumulated to potentially toxic levels in a relatively large and undifferentiated protoeukaryote dependent on the Emden-Meyerhof pathway for the production of ATP (Fig. 3). In support of this conjecture we may note that the giant amoeba *Pelomyxa palustris*, which lacks mitochondria but contains respiratory, endosymbiotic bacteria⁵⁷⁻⁵⁹, uses the Emden-Meyerhof pathway to supply its energy needs, produces lactic acid but has a requirement for oxygen⁶⁰, presumably for its endosymbiotic partners⁵⁸. *P. denitrificans* could serve in such a role, since it can utilise both lactic acid and ethanol as sources of energy and carbon⁶¹.

The most conspicuous morphological features of the mitochondrion are the invaginations of the inner membrane, termed cristae. By contrast the plasma membrane of *P. denitrificans* does not project into the interior of the cell, but remains applied to the internal surface of the cell wall^{62,63}. Presumably the development of cristae would be associated with the progressive specialisation of the aerobic bacterium resembling *P. denitrificans* essentially into a generator of ATP by oxidative phosphorylation. As this bacterium assumed its new role as a mitochondrion, the plasma membrane, as the site of oxidative phosphorylation, would increase in importance relative to the bacterial cytoplasm, which, as the site of redundant metabolic activities, would decrease in importance. Reflecting these changes, we could expect an expansion in the surface area of the plasma membrane, and a contraction in the volume of the cytoplasm, thus leading to the development of cristae.

Integration

By endowing the ancestor of the mitochondrion with the properties of the present-day bacterium *P. denitrificans*, we have provided a plausible account of the evolutionary origin of the inner mitochondrial membrane from the bacterial

plasma membrane. The major changes involved in this transition are: first, the loss of redundant components of the bacterial respiratory chain and, second, a modification in the transport properties of the bacterial plasma membrane, so that effective biochemical communication is attained between the mitochondrial matrix and the rest of the cell.

Many of the components of the mitochondrion that we believe to have come from the ancestral bacterium are now coded for in the nucleus and not in the mitochondrion^{64,65}. This implies that gene transfer has occurred during the evolution of the mitochondrion from a *Paracoccus*-like ancestor. Support for this conclusion may well come from comparing the amino acid sequence of the soluble cytochrome cytochrome *c* from *P. denitrificans* (when it is published) with the amino acid sequence of mitochondrial cytochrome *c*.

The evidence provided here lends support to the endosymbiotic theory, firstly, by removing some of the objections raised by previous authors⁴⁰, concerning the affinity of bacterial and mitochondrial respiratory chains and, secondly, by offering a feasible evolutionary transition from a bacterial plasma membrane to the inner mitochondrial membrane. This evidence on its own is, however, not incompatible with the non-symbiotic evolutionary origin of the mitochondrion proposed by Raff and Mahler⁴⁰, since their theory considers features common to bacteria and mitochondria to be "retained primitive states"⁸⁶. Furthermore, the changes which we have described for an evolutionary origin of the inner mitochondrial membrane from the plasma membrane of an aerobic bacterium resembling *P. denitrificans* would also apply to an evolutionary origin of the inner mitochondrial membrane from the plasma membrane of an aerobic protoeukaryote possessing a respiratory system similar to that of *P. denitrificans*. Having noted these points, however, we agree with Taylor⁶⁷ that it is "unrealistic to discuss the origin of mitochondria independently from that of chloroplasts". Thus it is in the context of the greater applicability of the endosymbiotic theory to explain the evolutionary origin of the eukaryotic cell as a whole⁶⁷ that we have chosen to compare *P. denitrificans* with the mitochondrion and to show how a relationship of limited advantage to both symbiotic partners could readily have developed into the present close relationship between a mitochondrion and its "host" cell.

We thank the Science Research Council for a research grant.

Received December 11, 1974; revised March 3, 1975.

- ¹ Margulis, L., *Origin of Eukaryotic Cells* (Yale University Press, New Haven, 1970).
- ² Davis, D. H., Doudoroff, M., Stanier, R. Y., and Mandel, M., *Int. J. syst. Bact.*, **19**, 375-390 (1969).
- ³ John, P., and Whatley, F. R., *Adv. bot. Res.* (in the press).

- ⁴ Asano, A., Imai, K., and Sato, R., *Biochim. biophys. Acta*, **143**, 477-486 (1967).
- ⁵ Murthy, P. S., and Brodie, A. F., *J. biol. Chem.*, **239**, 4292-4297 (1964).
- ⁶ Imai, K., Asano, A., and Sato, R., *Biochim. biophys. Acta*, **143**, 462-476 (1967).
- ⁷ Gel'man, N. S., Lukoyana, M. A., and Ostrovskii, N. D., *Respiration and Phosphorylation of Bacteria* (Plenum, New York, 1967).
- ⁸ Smith, L., in *Biological Oxidations* (edit. by Singer, T. P.), 55-122 (Interscience, New York, 1968).
- ⁹ Forget, P., and Pichnoty, F., *Annls Inst. Pasteur, Paris*, **108**, 364-377 (1965).
- ¹⁰ Doelle, H. W., *Bacterial Metabolism* (Academic, New York, 1969).
- ¹¹ Wilkinson, B. J., Morman, M. R., and White, D. C., *J. Bact.*, **112**, 1288-1294 (1972).
- ¹² Parsons, D. F., Williams, G. R., Thompson, W., Wilson, D., and Chance, B., in *Mitochondrial Structure and Compartmentation* (edit. by Quagliariello, E., Papa, S., Slater, E. C., and Tager, J. M.), 29-70 (Adriatica Editrice, Bari, 1967).
- ¹³ Girard, A. E., *Can. J. Microbiol.*, **17**, 1503-1508 (1971).
- ¹⁴ Getz, G. S., Bartley, W., Stirpe, F., Notton, B. M., and Renshaw, A., *Biochem. J.*, **83**, 181-194 (1962).
- ¹⁵ Crane, F. L., in *Biochemistry of Quinones* (edit. by Morton, R. A.), 183-206 (Academic, New York, 1965).
- ¹⁶ Scholes, P. B., and Smith, L., *Biochim. biophys. Acta*, **153**, 363-375 (1968).
- ¹⁷ Shipp, W. S., *Archs Biochem. Biophys.*, **150**, 482-488 (1972).
- ¹⁸ Shipp, W. S., *Archs Biochem. Biophys.*, **150**, 459-472 (1972).
- ¹⁹ Dutton, P. L., and Wilson, D. F., *Biochim. biophys. Acta*, **346**, 165-212 (1974).
- ²⁰ Kamen, M. D., and Horio, T., *A. Rev. Biochem.*, **39**, 673-700 (1970).
- ²¹ Erickson, S. K., *Biochim. biophys. Acta*, **245**, 63-69 (1971).
- ²² Kamen, M. D., and Vernon, L. P., *Biochim. biophys. Acta*, **17**, 10-22 (1955).
- ²³ Scholes, P. B., McLain, G., and Smith, L., *Biochemistry*, **10**, 2072-2075 (1971).
- ²⁴ Timkovich, R., and Dickerson, R. E., *J. molec. Biol.*, **79**, 39-56 (1973).
- ²⁵ Yamanaka, T., and Okunuki, K., *J. biol. Chem.*, **239**, 1813-1817 (1964).
- ²⁶ Yamanaka, T., *Space Life Sci.*, **4**, 490-504 (1973).
- ²⁷ Smith, L., Newton, N., and Scholes, P. B., in *Hemes and Hemoproteins* (edit. by Chance, B., Estabrook, R. W., and Yonetani, T.), 395-403 (Academic, New York, 1966).
- ²⁸ Lam, Y., and Nicholas, D. J. D., *Biochim. biophys. Acta*, **180**, 459-472 (1969).
- ²⁹ Newton, N., *Biochim. biophys. Acta*, **185**, 316-331 (1969).
- ³⁰ Scholes, P. B., and Mitchell, P., *J. Bioenerg.*, **1**, 309-323 (1970).
- ³¹ Beatrice, M. C., and Chappell, J. B., *Biochem. Soc. Trans.*, **2**, 151-153 (1973).
- ³² Lawford, H. G., and Haddock, B. A., *Biochem. J.*, **136**, 217-220 (1973).
- ³³ Downs, A. J., and Jones, C. W., *Biochem. Soc. Trans.*, **2**, 526-529 (1974).
- ³⁴ Mitchell, P., *FEBS Symp.*, **28**, 353-370 (1972).
- ³⁵ Moyle, J., and Mitchell, P., *Biochem. J.*, **105**, 1147-1162 (1973).
- ³⁶ John, P., and Hamilton, W. A., *FEBS Lett.*, **10**, 246-248 (1970).
- ³⁷ John, P., and Hamilton, W. A., *Eur. J. Biochem.*, **23**, 528-532 (1971).
- ³⁸ Jones, C. W., Erickson, S. K., and Ackrell, B. A. C., *FEBS Lett.*, **13**, 33-35 (1971).
- ³⁹ Walter, P., Lardy, H. A., and Johnson, D., *J. biol. Chem.*, **242**, 5014-5018 (1967).
- ⁴⁰ Raff, R. A., and Mahler, H. R., *Science*, **177**, 575-582 (1972).
- ⁴¹ Fewson, C. A., and Nicholas, D. J. D., *Biochim. biophys. Acta*, **48**, 208-210 (1961).
- ⁴² Pichinoty, F., *Annls Inst. Pasteur, Paris*, **109**, 248-255 (1965).
- ⁴³ Pichinoty, F., and D'Ornano, L., *Biochim. biophys. Acta*, **52**, 386-389 (1961).
- ⁴⁴ Ferguson, S. J., John, P., Lloyd, W. J., Radda, G. K., and Whatley, F. R., *Biochim. biophys. Acta*, **357**, 457-461 (1974).
- ⁴⁵ Tait, G. H., *Biochem. Soc. Trans.*, **2**, 657-658 (1974).
- ⁴⁶ Hamilton, W. A., *Adv. mic. Physiol.*, **12**, 1-53 (1975).
- ⁴⁷ Chappell, J. B., and Haarhoff, K. N., in *Biochemistry of Mitochondria* (edit. by Slater, E. C., Kaniuga, Z., and Wojtczak, L.), 77-91 (Academic, New York, 1967).
- ⁴⁸ Mitchell, P., in *Organization and Control in Prokaryotic and Eukaryotic Cells* (edit. by Charles, H. P., and Knight, B. C. J. G.), 121-166 (Cambridge University Press, Cambridge, 1970).
- ⁴⁹ Crompton, M., Palmieri, F., Capano, M., and Quagliariello, E., *Biochem. J.*, **142**, 127-137 (1974).
- ⁵⁰ Postma, P. W., and Van Dam, K., *Biochim. biophys. Acta*, **249**, 515-527 (1971).
- ⁵¹ Visser, A. S., and Postma, P. W., *Biochim. biophys. Acta*, **298**, 333-340 (1973).
- ⁵² Ghei, O. K., and Kay, W. W., *FEBS Lett.*, **20**, 137-140 (1972).
- ⁵³ Lawford, H. G., and Williams, G. R., *Biochem. J.*, **123**, 571-577 (1971).
- ⁵⁴ Chappell, J. B., *Br. med. Bull.*, **24**, 150-157 (1968).
- ⁵⁵ Klingenberg, M., *Essays in Biochemistry*, **6**, 119-159 (1970).
- ⁵⁶ Lehninger, A. L., *The Mitochondrion* (Benjamin, New York, 1964).
- ⁵⁷ Daniels, E. W., Breyer, E. P., and Kudo, R. R., *Z. Zellforsch. mikrosk. Anat.*, **73**, 367-383 (1966).
- ⁵⁸ Leine, M., Schweikhardt, F., Blaschke, G., König, K., and Fischer, M., *Biol. Zbl.*, **87**, 567-591 (1968).
- ⁵⁹ Chapman-Andresen, C., *A. Rev. Microbiol.*, **25**, 27-48 (1971).
- ⁶⁰ Andresen, N., Chapman-Andresen, C., and Nilsson, J. R., *C. r. Trav. Lab. Carlsberg*, **36**, 285-317 (1968).
- ⁶¹ Vogt, M., *Archs Mikrobiol.*, **50**, 256-281 (1965).
- ⁶² Kocur, M., Martinec, T., and Mazanec, K., *Antonie van Leeuwenhoek*, **34**, 19-26 (1968).
- ⁶³ Scholes, P. B., and Smith, L., *Biochim. biophys. Acta*, **153**, 350-362 (1968).
- ⁶⁴ Sherman, F., and Stewart, J. W., *A. Rev. Genet.*, **5**, 257-296 (1971).
- ⁶⁵ Tzagoloff, A., Rubin, M. S., and Sierra, M. F., *Biochim. biophys. Acta*, **301**, 71-104 (1973).
- ⁶⁶ Uzzell, T., and Spolsky, C., *Science*, **180**, 516-517 (1973).
- ⁶⁷ Taylor, J. F. R., *Taxon*, **23**, 229-258 (1974).
- ⁶⁸ Kronick, P., and Hill, G. C., *Biochim. biophys. Acta*, **368**, 173-180 (1974).
- ⁶⁹ Chance, B., and Williams, G. R., *J. biol. Chem.*, **217**, 383-393 (1955).

letters to nature

Search for continuous gravitational radiation

We have given¹ results of a search for short pulses of gravitational radiation carried out with two gravitational radiation detectors operating in coincidence over a frequency range from 650 to 1,450 Hz. Detectors of that type may also be used within this frequency region to set upper limits to fluxes of gravitational radiation of either continuous or incoherent waveform. The

latter may include pulses which occur at a high rate but are too small to be detected individually. We describe here a preliminary experiment designed to search for any such signals in a general way.

If the expected signal waveform is known there are specialised methods which can optimise the sensitivity of such an experiment. For example a continuous repetitive waveform of known repetition rate and wave shape, buried in the noise from a detector, can be recovered with high sensitivity by cross correlation against a reference waveform of the same characteristics;

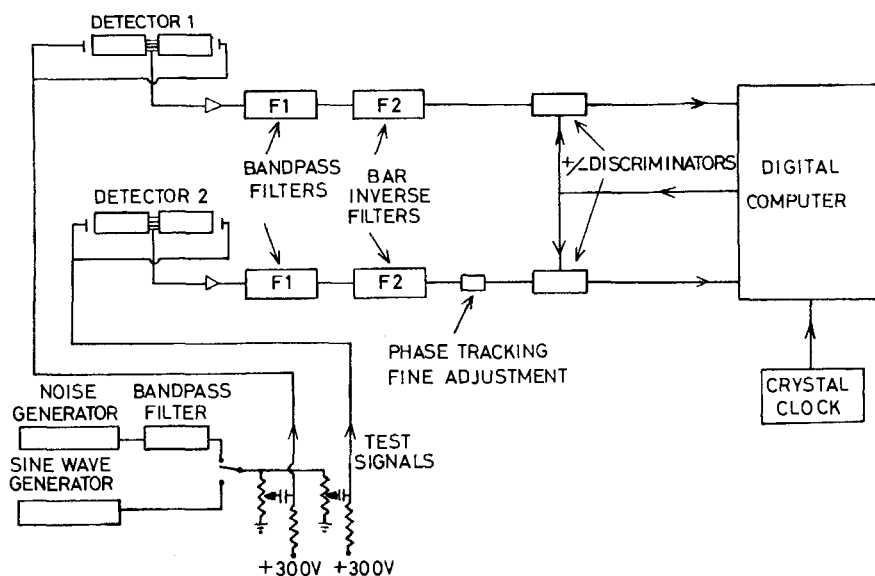


Fig. 1 Simplified block diagram of the cross correlation experiment. The sine wave and noise generators shown are used for calibration. F2 is a special filter with a null in its response at the resonant frequency of the bar and has the effect of giving an approximately flat non-resonant response to the whole system within the bandwidth of the bandpass filter F1.

or a monochromatic continuous signal can be detected by means of a Fourier transform technique. In a search for a signal of unknown type and waveform from a gravitational wave detector in the presence of noise, however, a more general technique is required. The technique used in this experiment was the cross correlation of the outputs from two gravitational wave detectors. This procedure allows signals common to both detectors in the presence of noise (which may contain a number of spurious detector resonances) to be recovered.

Although our search was sensitive to gravitational radiation of several different waveforms, for simplicity in this preliminary experiment we tested our apparatus and set upper limits for two types of radiation: first, continuous monochromatic radiation as may be produced by a rapidly rotating neutron star; and second, broadband gravitational radiation.

Previous searches for gravitational waves of monochromatic and broadband type have been made at very low frequencies, although a search for monochromatic radiation from pulsars at frequencies up to 125 Hz has been reported². An upper limit to broadband radiation has also been set by a laser interferometer gravitational radiation detector operating above 1 kHz (ref. 3). Our experiment sets a more sensitive upper limit for both broadband and monochromatic radiation in the frequency region around 1 kHz.

Each of our detectors consists of two separate aluminium masses linked by piezoelectric transducers to monitor changes in separation, this arrangement giving relatively high coupling between the mechanical and electrical systems. The signal from the transducers passes to a very low noise field effect transistor preamplifier and then through filters F1 and F2 (Fig. 1). The overall bandwidth was set at 160 Hz, centred on 985 Hz, this being estimated as the optimum bandwidth for the detection of white noise signals. This is narrower than the optimum bandwidth for a pulse search¹.

The signals from the filter F2 represent the forces acting on the detectors and are further processed and then cross correlated by a small on line computer. During most of our runs the polarity of the signals from one of the preamplifiers was reversed periodically and data for reversed and normal operation were recorded separately. This procedure is a useful test for any electronic coupling between the signals from the two detectors occurring at any point in the whole system after the preamplifiers. The recorded data were subsequently combined taking account of the polarity reversal.

Cross correlation entails the multiplication of the signal from one detector $x(t)$ by the signal from the other detector delayed by a time τ , $(y(t-\tau))$, and the cross correlation function

$R(\tau)$ for this delay is the average value of the product over the time of the experiment T .

$$R(\tau) = (1/T) \int_0^T x(t)y(t-\tau)dt$$

If both signals are sampled every Δt seconds for input to a computer the correlation function R_n becomes

$$R_n = (1/K') \sum_{k=1}^{K'} x(k\Delta t)y(k\Delta t - n\Delta t), n \leq K'$$

and the variation of R_n with n can give information as to the waveform of any signal common to the two detectors. In conditions of low signal-to-noise ratio, little information is lost^{4,5} if the correlation is performed on polarity alone. The filtered signals from the detectors are sampled and have their sign determined at regular intervals by a discriminator-latch circuit controlled through our computer by a crystal clock. A '1 bit' cross correlation on the data is then computed. The signs of the signals at each sampling time are recorded and a number of successive sign samples from the first detector are stored in order, this store being updated each time a new sample is taken. The current sample from the second detector is compared with each of the samples from the first one and the result (+1 if they are of the same sign, -1 if they are different) is added to the results for previous samples. In this way a number which is related to the cross correlation function is found as a function of delay between the detectors, and these numbers are displayed as our delay curves.

Our maximum possible sampling rate was limited by the length of programme to 2 kHz and with this 17 correlation points 0.5 ms apart were obtained. To record the delay curve with more detail in the region near zero delay, some runs were made in which the data were recorded on an analogue tape recorder and subsequently slowed down by a factor of 4 for analysis off line.

Calibration was carried out by applying signals of known waveform to the capacitive endplates of both detectors simultaneously. Both broadband noise and sine waveforms off the resonant frequencies of the detectors were used, the energy deposited per unit time in the detectors being measured in the first case and the force applied known in the latter case. From these the amplitudes of the delay curves were found in

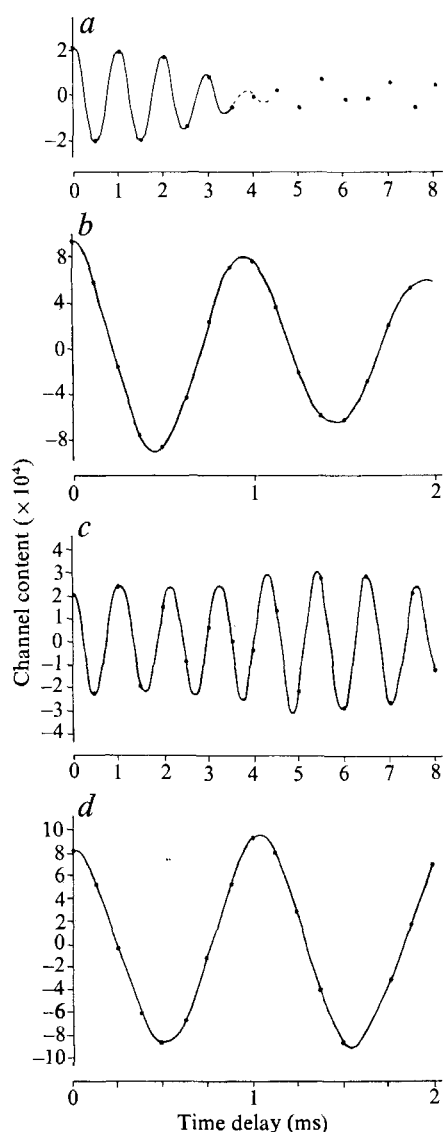


Fig. 2 Delay curves for calibration force waveforms applied to the detectors by means of capacitive endplates; *a* and *b* are the curves for an applied broadband noise waveform. *a*, Obtained by on-line analysis of the data; *b*, off-line analysis of the same data recorded and slowed down by a factor of 4. *c* and *d* are equivalent curves for a sinusoidal calibration waveform at 910 Hz.

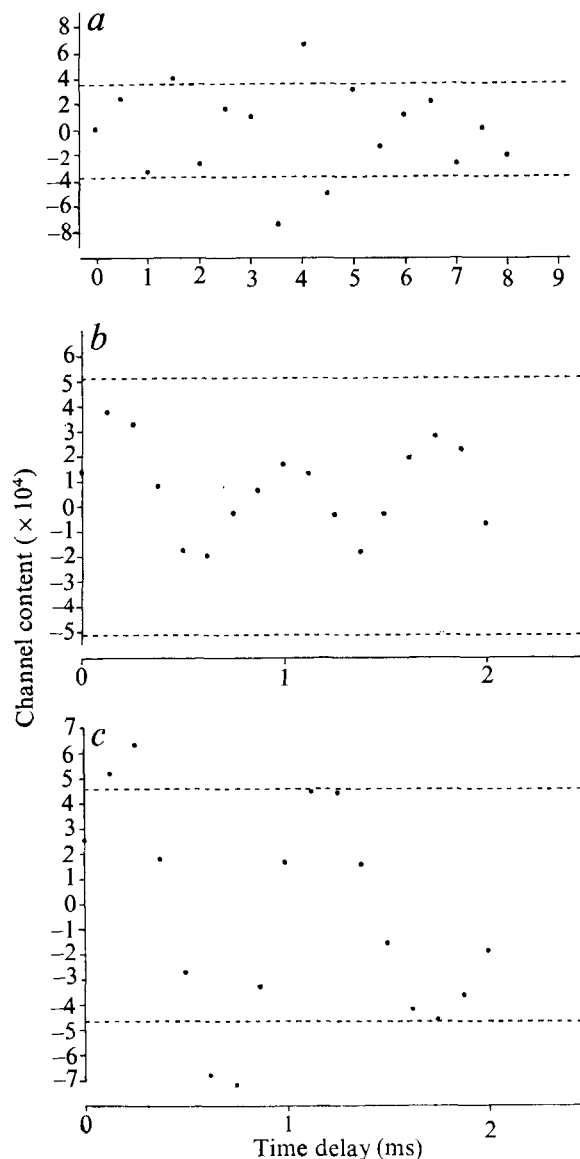
terms of gravitational radiation flux. The flux was calculated by the standard formulae⁶. Figure 2*a* and *b* shows the delay curves (measured over 52 min) for a broadband white noise signal equivalent to a gravitational wave flux of $4.3 \times 10^4 \text{ W m}^{-2} \text{ Hz}^{-1}$ averaged over the system bandwidth. The shape of the curves is a characteristic decaying sinusoid the fall time of which is related to the system bandwidth of 160 Hz. Figure 2*c* and *d* shows the equivalent response over 52 min for a sine wave test signal at 910 Hz, the corresponding gravitational wave flux being $2.7 \times 10^7 \text{ W m}^{-2}$.

The delay curve obtained in a run lasting 90 h with no calibration signals applied is shown in Fig. 3*a*, with delays up to 8 ms. Expanded delay curves obtained by analysis of recorded data from two interleaved parts of a separate 24-h run are shown in Fig. 3*b* and *c*. To assess the significance of the delay curves it is necessary to know the magnitude of the fluctuations which may be expected to arise purely from the uncorrelated noise in the detectors, and in the rest of the system. This may be estimated, but we have also determined it empirically from independent measurements of the delay curves for a large

number of short experiments. The dotted lines in the figures indicate the root mean square deviation from the mean expected from uncorrelated data, found in this way. In making a comparison with the experimental curves, allowances have to be made for the fact that successive points in any one delay curve are correlated among themselves over a time scale of about 3 ms because of the bandwidth of the system. The experimental data do not show to any significant extent the waveforms in the test curves of Fig. 2, do not show any persistent pattern from run to run and are in fact quite consistent with completely independent noise inputs from the two detectors. The curve with the largest amplitude near zero delay is that in Fig. 3*c* but the most extreme point corresponds to only 1.4 standard deviations from the mean.

We conclude that the data give no indication of correlated signals from the two detectors. From the measured properties of the detectors it is possible to set upper limits to fluxes of gravitational radiation signals of various types, and as mentioned earlier we can set limits on wideband gravitational flux and on monochromatic radiation in our frequency band.

Fig. 3 Delay curves with no calibration forces applied to the detectors. *a*, The result of an on-line analysis of a 90 h experiment (September 6–10, 1974). *b* and *c* were obtained by off-line analysis of data from two parts of a separate 24 h experiment. (August 6–7, 1974), slowed down by a factor of 4 before analysis.



From the 90-h run we set an upper limit at a level of 3 standard deviations of about $2 \times 10^3 \text{ W m}^{-2} \text{ Hz}^{-1}$ for wide-band unpolarised gravitational radiation and an upper limit, again at a level of 3 standard deviations, of about $1 \times 10^6 \text{ W m}^{-2}$ for a continuous sinusoidal signal in the frequency range of 910–1,070 Hz, assuming the radiation is unpolarised and incident normal to our detectors for the total length of the run in each case.

Our corresponding upper limit for isotropic wideband unpolarised radiation⁷ is about $4 \times 10^3 \text{ W m}^{-2} \text{ Hz}^{-1}$ and for an unpolarised monochromatic source is about $2 \times 10^6 \text{ W m}^{-2}$, the exact value depending to some extent on the relative orientation of the source and the detectors.

This work has some relevance to the interpretation of the pulse experiments of J. Weber. Observations from several groups seems to be in conflict with Weber's results but we feel that because of the differing signal processing techniques used it has not been clear that an explanation in terms of a very large flux of small gravitational radiation pulses is completely ruled out. But a flux consisting of 5,000 or more pulses per day depositing an aggregate energy of about 500 times the mean thermal energy per mode in one of our detectors (500 kT), would have been observed at the three standard deviation level in the present experiment, independent of details of pulse duration and waveform. If Weber's coincidences were the result of pulses corresponding to energies of 0.2 kT or less in his detectors our estimate of his experimental sensitivity, as well as the more detailed analysis of Levine and Garwin⁸, suggests that the total energy flux required would be greater than the limit above.

Our negative result thus provides evidence against the hypothesis that the signals reported by Weber are caused by a large flux of very small pulses.

We acknowledge the financial support of the University of Glasgow and of the Science Research Council and thank Professor J. C. Gunn for his interest and encouragement.

J. HOUGH
J. R. PUGH
R. BLAND
R. W. P. DREVER

Department of Natural Philosophy,
University of Glasgow, UK

Received December 31, 1974; revised February 24, 1975.

¹ Drever, R. W. P., Hough, J., Bland, R., and Lessnoff, G. W., *Nature*, **246**, 340–344 (1973).

² Mast, T. S., Nelson, J. E., and Saarloos, J. A., *Astrophys. J. Lett.*, **187**, L49–L51 (1974).

³ Forward, R. L. (see Burke, W. L.), *Phys. Rev.*, **8D**, 1030–1035 (1973).

⁴ Weinreb, S., *Tech. Rep.*, 412 (MIT, 1963).

⁵ Davies, R. D., Ponsonby, J. E. B., Pointon, L., and Jager, G. de., *Nature*, **222**, 933–937 (1969).

⁶ Press, W. H., and Thorne, K. S., *A. Rev. Astr. Astrophys.*, **10**, 335–374 (1972).

⁷ Misner, C. W., Thorne, K. S., and Wheeler, J. A., *Gravitation*, equation 37.21 (Freeman, San Francisco, 1973).

⁸ Levine, J. L., and Garwin, R. L., *Phys. Rev. Lett.*, **33**, 794–797 (1974).

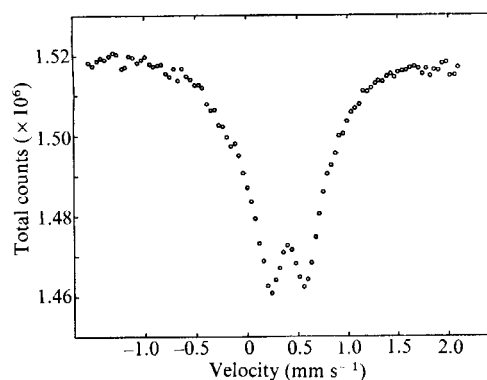


Fig. 1 Mössbauer absorption spectrum of ^{57}Fe in thoro-steenstrupine. Other spectra, spanning velocities up to 4.0 mm s^{-1} showed no evidence of any Fe^{2+} ions.

bauer absorption spectrum of a 100 mg cm^{-2} sample of thoro-steenstrupine powder. The spectrum is consistent with the iron appearing as Fe^{3+} in two different sites, both with octahedral symmetry². Most of the Fe^{3+} ions occupy a M_2 site with an isomer shift (relative to metallic iron) of $+0.40 \pm 0.03 \text{ mm s}^{-1}$ and a quadrupole splitting of $0.34 \pm 0.03 \text{ mm s}^{-1}$; a very small amount (less than 5%) of iron atoms occupying a more distorted M_1 site with an isomer shift of $+0.37 \pm 0.06 \text{ mm s}^{-1}$ and a quadrupole splitting of $0.95 \pm 0.07 \text{ mm s}^{-1}$. The consistency of these parameters with the systematics for other minerals²—which allowed the site assignments—and the relatively large intensity of the absorption lines show that the Mössbauer pattern is not affected by the radiation damage that precluded a X-ray diffraction study of the same sample, although thorium-free thoro-steenstrupine is known to crystallise with the apatite structure (J. Metcalf, personal communication). This would be expected, since quadrupole splitting and isomer shift depend primarily on the immediate surroundings of the iron atom. Moreover, if the crystalline environment around the metal ion were damaged appreciably, a more complex pattern with strong interactions could be expected⁴.

I thank J. Metcalf for the loan of the sample and informal teachings of mineralogy, and the Institute of Physics, University of Aarhus, Denmark, for the use of the Mössbauer equipment.

E. J. ANSALDO*

PO Box 708, Princeton, New Jersey 8340

Received January 22, 1975.

*Present address: Physics Department, University of Saskatchewan, Saskatoon, S7N/OWO, Canada.

¹ de Coster, M., Pollack, H., and Amelinckx, S., *Phys. Stat. Solids*, **3**, 283 (1963).

² Bancroft, G. M., Burns, R. G., and Stone, A. J., *Geochim. cosmochim. Acta*, **32**, 547 (1968).

³ Greenwood, N. N., and Gibb, T. C., *Mössbauer Spectroscopy* (Chapman and Hall, London, 1971).

⁴ Matsui, K., Hasiguti, R. R., and Onodera, H., *Rad. Effects*, **8**, 195 (1971).

Mössbauer absorption in a metamict mineral

THE technique and application of ^{57}Fe Mössbauer spectroscopy to minerals have been discussed fully^{1–3} in the context of the determination of oxidation states, coordination numbers, and site symmetry and population. This report shows, with the particular example of metamict thoro-steenstrupine $\{\text{Na}_2\text{Ce}(\text{Mn}, \text{Fe}, \text{Ta})(\text{La}, \text{Th}, \dots)(\text{Si}, \text{P})\text{O}_4\}_3\text{H}_2$, that the Mössbauer technique can yield structural information even when X-ray diffractometry is not possible because of the destruction of the lattice ordering by the damage produced by radioactive decays.

The Mössbauer spectrometer used was of conventional design, using a ^{57}Co in copper source and metallic iron calibration foils, as described previously³. Figure 1 shows the Möss-

Mössbauer spectroscopy of Chinese glazed ceramics

FOR over 1,000 yr the Chinese have manufactured glazed ceramic objects renowned for their aesthetic qualities. The colour of a glaze is generally achieved by the incorporation of transition metal ions¹ of which iron is the most important, being used in at least nine recognised glaze types, ranging in colour from pale blue, through green, to yellow, brown and red. Although reflectance spectra of the glazes allow a simple division into types expected to contain Fe^{2+} and Fe^{3+} ions, a study of the environment of the iron in each type is of interest. Such data may throw light on the technology of production

Table 1

Glaze type	Colour	Isomer shift*		Quadrupole splitting	
		Fe ²⁺	Fe ³⁺	Fe ²⁺	Fe ³⁺
Chun	Pale blue	1.08		1.04	
Celadon	Pale green	1.1	0.26	2.55	1.29
		0.82†		1.29†	
Celadon	Pale green	1.12		1.81	
		0.69†		1.38†	
Northern Celadon	Olive green		0.37		1.16
Chien (surface)‡	Rust brown		0.30		1.3
(interior)	Dark grey	1.16		2.16	
Mirror Black	Black	1.08	0.30	2.02	1.29

All measurements made at 300 K; those marked † also made at 77 K. Units are mm s⁻¹.

*With respect to metallic iron.

†These values may be tetrahedral species.

‡Also gave the spectrum of haematite.

of the glazes and permit archaeological distinctions between apparently similar glazes, for example, the famous pale green Celadons.

Here, I report results from a preliminary study of the Mössbauer spectra of the iron in a selection of Chinese glazes. This technique has already been applied to the elucidation of the structural role of iron in experimental silicate, phosphate and borate glasses^{2,3}, often in conjunction with optical and electron spin resonance spectroscopic and magnetic susceptibility measurements. In glasses, Fe is generally coordinated to O atoms, although traces of S or Se may coordinate to the Fe in glass, with a marked effect on the spectrum. In silicate glasses the evidence suggests a fourfold or sixfold coordination for Fe³⁺ (that is, substituting for Si⁴⁺ in SiO₄ tetrahedra, or for such ions as Na⁺), whereas Fe²⁺ is probably always in a sixfold coordination². Since the ligand field and distribution of energy levels change with coordination geometry the visible spectrum is strongly influenced by the site geometry of the ions. The Mössbauer spectrum is modified by the electronic charge density, by the electric field gradient, and by any magnetic field at the nucleus. These are manifested as a shift in the absorption energy (isomer shift), a doublet (quadrupole) splitting, and a splitting into hyperfine structure, respectively. Measurement of the isomer shift provides information on the oxidation state and coordination of the iron. Values of the quadrupole splitting indicate the degree of distortion from octahedral symmetry, and moreover help to identify a particular environment, being characteristic for particular compounds. Excess linewidth, beyond that found for crystalline iron compounds, gives a measure of the spread of differing environments for the Fe atoms. The presence of phases with internal magnetic fields may be established from hyperfine structure in the spectrum. Mössbauer spectra can decide various questions that arise in glaze studies: is the range of colour from blue to green in 'reduced' glazes caused by changes in the Fe²⁺/Fe³⁺ ratio, or by changes in the Fe²⁺ environment? Is the yellow in Imperial Yellow (a lead glaze) caused by Fe³⁺ (d-d) transitions, by colloidal Fe₂O₃, or by Fe³⁺ charge transfer bands? What glazes owe their colour to Fe²⁺-Fe³⁺ charge transfer spectra? The Chien glaze (opaque rusty-brown) is believed to be caused by the formation of Fe₂O₃; how well controlled is the growth and precipitation of this phase? Is Fe₂O₃ used to produce the 'Coral Red' type of glaze? How is the 'Tea Dust' (green-brown) glaze produced?

The samples consisted of a Chun glaze (pale blue), two Sung period Celadons, a Northern Celadon (olive green), an Imperial (Ming) Yellow, an interior and surface sample from a Chien glaze, and a Mirror Black glaze. Several important types are not represented; the samples were chosen by their availability for destructive analysis. About 100 mg of fine powder was obtained by grinding off the glaze using a diamond wheel with the object immersed in water to prevent any atmospheric oxidation from the heat evolved by friction. Mössbauer

spectra were recorded from 20–100 mg samples enclosed in thin plastic. The Fe content varies from 5% (Chien glaze) to less than 1% (reduced glazes), and several days were necessary to achieve satisfactory spectra for some samples. A conventional constant acceleration spectrometer was used in the transmission mode. Typically, 1–2 × 10⁶ counts were accumulated in each channel, with a maximal absorption in the range 0.5–5%. Only one sample (Chien) was studied at low temperature (77 K). Most of the spectra could be satisfactorily fitted with a combination of Lorentzian curves characteristic for quadrupole doublets.

The results of analysis of the spectra are presented in Table 1 and an example of the spectra obtained, together with computer-fitted component curves, is displayed in Fig. 1. In general, the

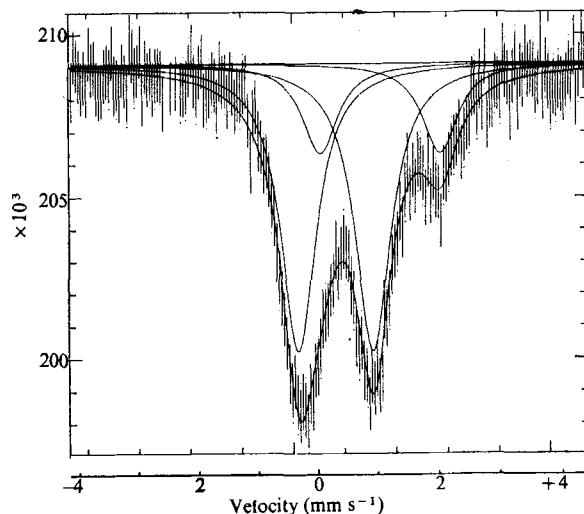


Fig. 1 Mössbauer spectrum of the Mirror Black glaze, showing its resolution into Fe²⁺ and Fe³⁺ doublets.

spectra could be interpreted as sums of Fe²⁺ and Fe³⁺ quadrupole split doublets (for details, see legend to Table 1). Measured linewidths are about twice that found in crystalline compounds. As is commonly found, it seems that sites giving larger splittings, within a given glaze, also have larger isomer shifts.

Chun glaze. The blue colour has been attributed to the formation of vivianite, as the glaze contains phosphorus¹. The quadrupole splitting does not, however, correspond to that for the mineral, nor to that for a phosphate glass. It is not evident whether the colour is caused by a modification of the characteristic 1 μm Fe²⁺ band, or by a radical change in the energy level structure.

Celadon glazes. Curve fitting to the weak and complex spectra is difficult, but nevertheless reveals unusual values for the isomer shift for Fe²⁺, the presence of tetrahedrally coordinated iron being indicated. Despite the close visual similarity of the glazes, the two Mössbauer spectra are quite distinct.

Northern Celadon. Assumed to be a more oxidised version of a Celadon glaze, this sample seems to be almost entirely Fe³⁺, with typical parameters.

Chien glaze. The 300 K spectrum shows mainly a typical Fe³⁺ doublet, with a small contribution from the six-line magnetic spectrum of haematite. At 77 K the spectrum is dominated by the magnetically split contribution. The relaxation of a magnetically split spectrum with temperature is characteristic of a temperature-dependent spin-lattice relaxation time or of a bulk phase with large surface area. Measurement of the magnetic splitting gives a magnetic field equal to that for haematite (α-Fe₂O₃). Haematite in the bulk phase does not show appreciable relaxation at 300 K, and it seems likely that the appearance of the Chien glaze is caused by the presence

of colloidal size (20–1,000 Å) particles of Fe_2O_3 (superparamagnetic behaviour). There is evidence for similar superparamagnetic behaviour of haematite in clays⁴. The production of such particles in a glass is of considerable intrinsic interest, and is also informative on the technical control of glaze production. The interior of the Chien glaze, which is dark grey, has a spectrum characteristic of Fe^{2+} , probably as a simple doublet. There may be a small contribution from magnetic phases (for example, Fe_2O_3 or Fe_3O_4) resolvable with improved signal-to-noise ratio. This suggests that the Chien glaze is first developed in a reducing atmosphere, and is then superficially oxidised. Such a process may be important in the production of colloidal Fe_2O_3 .

Mirror Black. This glaze has a very uniform reflectance spectrum in the visible region, and is produced by adding cobalt and manganese to a glaze containing 5–10% Fe. The visible spectrum may be a superposition of the absorption spectra of Fe^{3+} , Co^{2+} , and Mn^{2+} ; or more complex equilibria may be involved, perhaps leading to charge-transfer spectra. In any case, it is interesting that the Mössbauer spectrum shows the presence of appreciable Fe^{2+} (of the order of 25%).

Imperial (Ming) Yellow. No spectrum was obtained because of the strong absorption of the lead content of the glaze.

Further work on Chinese glazes must await the construction of a Mössbauer spectrometer working in the reflection mode, and capable of obtaining complex spectra from samples containing less than 1% Fe. This would enable a set of representative samples to be examined without destroying the source. This work is now in progress.

I thank Dr A. G. Maddock for his help and interest, and Professor E. T. Hall for his encouragement. Some of the sherds were kindly donated by Sir Harry Garner.

R. E. M. HEDGES

Research Laboratory for Archaeology
and the History of Art,
Oxford University,
6 Keble Road,
Oxford, UK

Received December 17, 1974; revised February 27, 1975.

¹ Hetherington, A. L., *Chinese Ceramic Glazes* (Cambridge University Press, 1948).

² Kurkjian, C. R., *J. Non-Cryst. Solids*, **3**, 157 (1970).

³ Taragin, H. F., Eisenstein, J. C., and Haller, J. *Phys. Chem. Glasses*, **13**, 149 (1972).

⁴ Van der Kraan, A. M., *J. Phys. theor. appl.*, **32**, Suppl. 1, C1, 1035 (1971).

Matrix synthesis and characterisation of dichromium

MATRIX isolation has been widely used to trap reactive species in the cavities of an inert host for subsequent spectroscopic observation¹. Occasionally, spectral features appeared which could not be ascribed to the single trapped species and had to be attributed to an aggregate. These bands were generally regarded as parasitic and their origin was not pursued. We have discovered that metal dimers can be readily formed in large quantities in matrix conditions and that the bands associated with these entities can be identified readily using a novel technique which makes use of the fact that the metal atoms being deposited are capable of diffusion either on the matrix surface or within a narrow region (the reaction zone) near the surface before its kinetic energy is dissipated sufficiently to immobilise it.

This process can be explained in terms of a kinetic model which assumes that at constant deposition rate of matrix material a steady state exists in the concentrations of the various species within the moving reaction zone. Metal dimers and higher aggregates form within the reaction zone to a much greater extent than indicated by a statistical analysis based on the matrix ratio. The dependence of the concentration of the various species on the rate of deposition of metal can then be

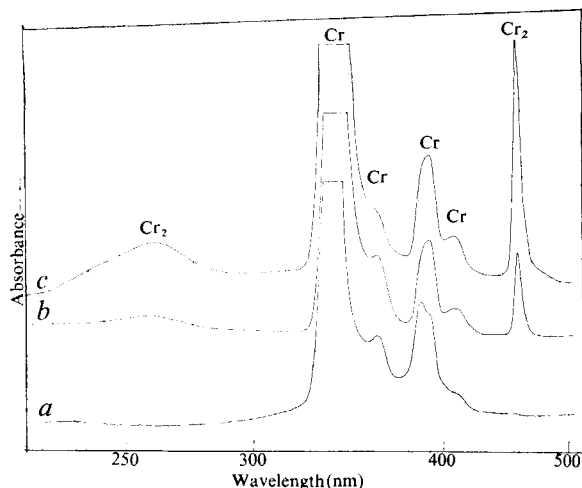


Fig. 1 A portion of the spectrum of chromium atoms and dichromium molecules in argon. *a*, Low metal flow; *b* and *c*, progressively higher metal flow.

calculated and compared with the observed dependence of the absorbance of bands on metal deposition rate.

Using this technique we have formed and identified dimers of V, Cr, Mn, Co, Ni, and Cu and observed their spectra in the ultraviolet and visible region. We use chromium to illustrate this technique.

Our experimental apparatus has been described elsewhere². The crucial aspect of this experiment is the deposition of metal at an accurately known and constant rate. A quartz crystal microbalance was used for this purpose³. Argon (Matheson) was deposited at approximately 2 mmol h⁻¹. Chromium (McKay, New York) was deposited from a Knudsen cell lined with boron nitride at various rates ranging from a few parts per thousand to a few per cent of the argon rate.

Figure 1 shows a portion of the spectrum of chromium in argon. As the rate of chromium metal deposition is increased the features at 260 and 455 nm grow. Figure 1*a* was obtained by depositing Cr into argon at such a slow rate that mainly chromium atoms are found in the matrix. This spectrum is nevertheless quite different from those reported by Mann and Broida⁴ and by D. Gruen (unpublished) which also differ from one another. Our experiences with this system suggest that the discrepancies are probably attributable to impurities and/or higher chromium aggregates in the earlier studies. In Table 1

Table 1 Frequencies and assignments for matrix isolated Cr and Cr_2 in argon

$\nu_{\text{Ar matrix}}$	ν_{gas} (Ref. 6)	$\Delta\nu = \nu_{\text{g}} - \nu_{\text{Ar}}$	Assignment	Species
29,400(vs)	$\begin{cases} 27,935 \\ 27,820 \\ 27,728 \end{cases}$	$\sim -1,600$	$a^7S \rightarrow y^7P^0$	Cr
26,900(w)	$\begin{cases} 24,971 \\ 25,010 \\ 25,089 \\ 25,206 \\ 25,359 \\ 25,548 \\ 25,771 \end{cases}$	$\sim -1,700$	$a^7S \rightarrow z^7F^0$	Cr
$\begin{cases} 25,800(s) \\ 25,500(s) \end{cases}$	$\begin{cases} 23,499 \\ 23,386 \\ 23,305 \end{cases}$	$\sim -2,200$	$a^7S \rightarrow z^7P^0$	Cr
24,300(vw) 38,500 22,000			?	Cr Cr_2 Cr_2

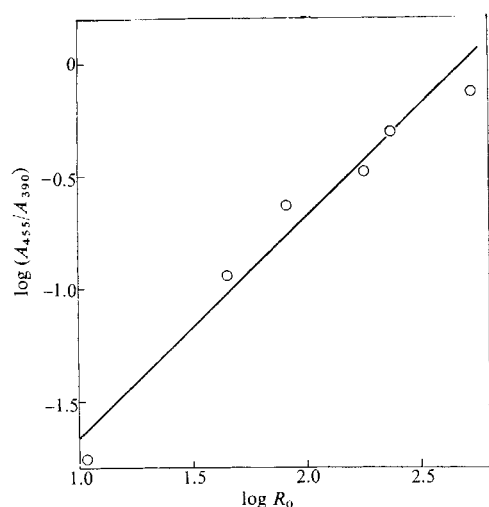
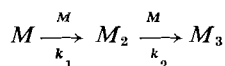


Fig. 2 A log-log plot of the ratio of the absorbance of a line attributed to Cr_2 to that of a Cr resonance absorption as a function of the chromium metal deposition rate at constant argon deposition rate.

we compare the frequencies of the lines measured for Cr in argon with those of the gaseous atom⁵. A blue matrix shift of approximately $2,000\text{ cm}^{-1}$ is observed for all the lines.

Unambiguous assignment of the broad band at 260 nm has not been made. We know, however, that at least the band at 455 nm belongs to Cr_2 . Consider the reaction



which we assume takes place in the reaction zone. At constant metal deposition rate R_0 a steady state exists in the concentrations of M , M_2 and M_3 given by the equations

$$dM/dt = R_0 - k_1 M^2 - k_2 M M_2 - rM = 0 \quad (1)$$

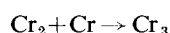
$$dM_2/dt = \frac{1}{2} k_1 M^2 - k_2 M M_2 - rM_2 = 0 \quad (2)$$

$$dM_3/dt = k_2 M M_2 - rM_3 = 0 \quad (3)$$

where r is related to the rate at which these species are frozen out and is proportional to the argon deposition rate. At low metal-deposition rates the ratio of dimer concentration to atom concentration is proportional to R_0 and the ratio of trimer concentration to atom concentration is proportional to R_0^2 (J. Hulse, E. P. K., M. M., and G. A. O., unpublished).

Figure 2 is a plot of $\log A_{455}/A_{390}$ as a function of $\log R_0$ together with a line of unit slope, where A_{455} and A_{390} are respectively the absorbances of the unknown 455-nm line and the 388–392-nm Cr atom doublet. The excellent correlation between the unit slope line and the experimental points suggests that the 455-nm line belongs to Cr_2 and not to a higher aggregate.

The apparent absence of Cr_3 could be a result of a low extinction coefficient, or a low k_2 value, or both. The latter reason may be a result of the activation energy required in the reaction



in which the Cr_2 bond may have to be weakened before the reaction can proceed. The kinetic result, incidentally, also suggests that Cr_2 is formed in or on the matrix rather than in the gas phase. This last fact is also borne out by the intriguing observation that for a given metal deposition rate the dimer-monomer ratio decreases as one increases the molecular weight

of the noble gas used to isolate them, with neon giving the most dimers and xenon the least. This is understood to be a consequence of the formation of Van der Waals' complexes between the metal and the noble gas molecule with the most polarisable noble gas atom forming the strongest complexes. A metal atom complexed in this fashion can either be construed to be quenched or at least its diffusion slowed down considerably.

We thank the National Research Council and the Research Corporation for support.

E. P. KÜNDIG
M. MOSKOVITS
G. A. OZIN

Lash Miller Laboratories and Erindale College,
University of Toronto, Toronto, Canada

Received February 4, 1975.

¹ Whittle, E., Dows, D. A., and Pimentel, G. C., *J. Chem. Phys.*, **22**, 1943 (1954).

² Kündig, E. P., Moskovits, M., and Ozin, G. A., *J. molec. Struct.*, **14**, 137 (1972).

³ Moskovits, M., and Ozin, G. A., *Appl. Spect.*, **26**, 481 (1972).

⁴ Mann, D. M., and Broida, H. P., *J. Chem. Phys.*, **55**, 84 (1971).

⁵ Moore, C. E., *Atomic Energy Levels*, National Bureau of Standards Circular 467.

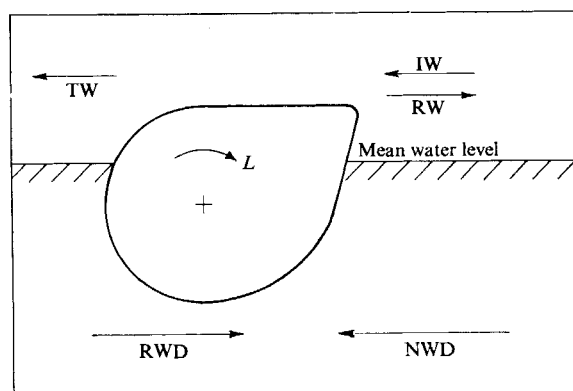
Characteristics of a rocking wave power device

THE possibility of extracting a large proportion (more than 80%) of the total wave power from water waves has been demonstrated experimentally¹ using a specially contoured rocking device. In considering the possibility of large scale power generation, it is necessary to understand how such a device would respond to the variable Atlantic ocean conditions where wave periods are between 7 s and 14 s for 99% of the time². We here present the results of initial investigations of the bandwidth of wave periods covered by Salter's¹ device.

The essential features of Salter's rocking shape (Fig. 1) are a circular rear section which has a constant displacement (and therefore does not transmit any energy) and a front section with a non-uniform radius. When it is matched to a wave of wavelength λ , the front section is designed to move so that it does not perturb the fluid motion of the incoming waves—which decays exponentially with depth, y , as $v_0 \exp(-2\pi y/\lambda)$ —and which thus does not reflect any energy. In principle, therefore, the only energy not absorbed by the device is that proportion which propagates underneath; from simple theory this would amount to $\exp(-4\pi d/\lambda)$ where d is the depth of immersion³.

A measure of the effectiveness of the matching is provided by the difference between the body velocity and the unperturbed fluid velocity along the submerged surface, s , in the direction normal to the surface. An estimate of the proportion of the

Fig. 1 The Salter cam¹. TW, Transmitted wave; IW, incident wave; RW, reflected wave; NWD, normal wave direction; RWD, reversed wave direction. L , load ($=T_0 K\theta$).



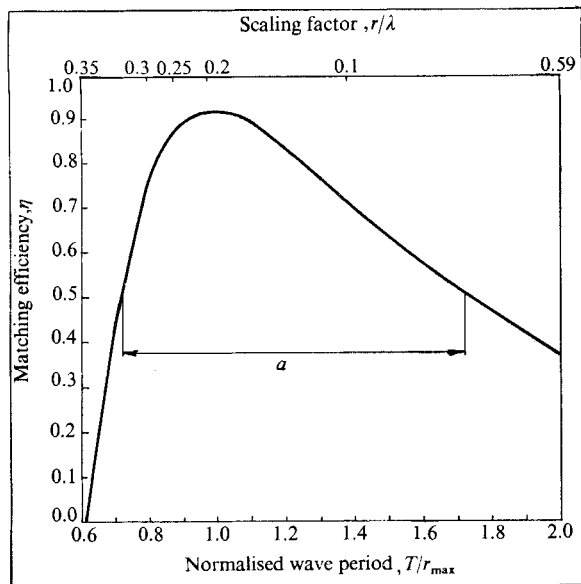


Fig. 2 Theoretical matching efficiency of a Salter cam. *a*, Half power bandwidth.

incident energy reflected or, more properly, scattered from this surface is given by

$$R = \int \overline{(v_n - u_n)^2} ds / \int \overline{u_n^2} ds \quad (1)$$

where v_n and u_n are the body velocity and unperturbed fluid velocity (normal to the surface), respectively, and the integrations are time averaged. Equation (1) has been evaluated for the Salter shape¹, oscillating sinusoidally, and the minimum value of R has been found for a range of wavelengths in terms of a characteristic dimension of the device, r , which is taken to be the back radius. The overall absorption efficiency, η , is defined as

$$\eta = (1 - R_{\min})[1 - \exp(-4\pi d/\lambda)] \quad (2)$$

where $[1 - \exp(-4\pi d/\lambda)]$, takes account of the proportion of the incident energy transmitted underneath the device. It is plotted in Fig. 2 against the normalised dimensions r/λ and normalised wave period. Efficiencies of over 90% are predicted for $0.16 < r/\lambda < 0.20$ and the half power bandwidth exceeds 2:1.

These are most encouraging theoretical results which imply that a device 50 m in diameter, tuned to a wave of period 10 s ($\lambda \approx 150$ m) would couple with at least 50% absorption efficiency to waves with periods of between 7 s and 17 s ($75 \text{ m} < \lambda < 450 \text{ m}$). We have tested these findings experimentally on a small scale in a wave tank.

The load on a device 10 cm in diameter, of the type described by Salter¹, was tuned to give maximum power output as measured by a calibrated dynamometer, from incident waves with periods, T , ranging between 0.38 and 1.0 s ($0.03 < r/\lambda < 0.22$). For periods longer than 1.0 s, assumptions of deep water conditions ceased to apply in our tank. Waves with periods of less than 0.38 s were attenuated significantly. Over the intermediate range, attenuation could be ignored and then the incident and reflected wave amplitudes, A and B , respectively, were calculated from the standing wave ratio, S , defined by:

$$S = (A - B)/(A + B)$$

where $(A - B)$ and $(A + B)$ are, respectively, the minimum and maximum amplitudes measured on the standing wave in front of the device. The amplitude, C , of the wave transmitted past the

device was also measured. The incident, reflected and transmitted powers were calculated (see ref. 3) from these amplitudes respectively:

$$(P_i, P_r, P_t) = (\rho g^2 T / 8\pi) (A^2, B^2, C^2)$$

and the proportion of power lost from the wave, W , is

$$W = [P_i - (P_r + P_t)] / P_i \\ = 1 - (B^2 + C^2) / A^2$$

W is plotted against T in Fig. 3a. This curve is very similar to the predicted efficiency curve (Fig. 2). We also obtained a direct

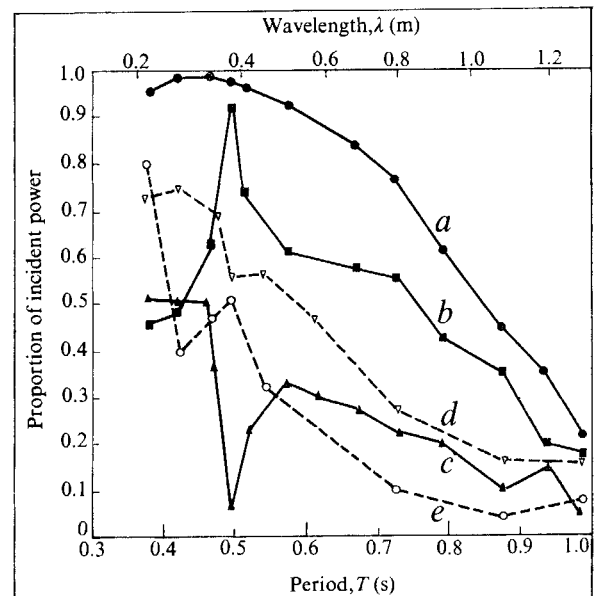


Fig. 3 Efficiency and losses for the Salter cam. *a*, W (tuned); *b*, η_g ; *c*, losses (tuned); *d*, W (free); *e*, W (fixed).

measurement of the power absorbed by the device at the dynamometer, and this is plotted (Fig. 3b) normalised to the incident power P_i , and corresponds to the actual efficiency of power extraction η_g . Though both curves peak at over 90%, the useful power output curve suggests that the device/load combination is resonant at $T=0.5$ s ($r/\lambda=0.13$) with a rapid fall off in efficiency to about 60% on either side. The difference between Fig. 3a and 3b represents the proportion of the incident power lost from the wave system because of turbulent and other nonlinear effects (Fig. 3c). Although small at the 'resonant' period (about 5%) these losses are, in general, surprisingly large, as high as 50% for the shorter periods.

Losses will be very important in practical systems and so they warrant further investigation. To assess the importance of the motion of the device in causing these losses, the experiments were repeated with the device either oscillating freely or held rigid (Fig. 3a and b). As no power can be extracted by the device in these cases, W can only represent system losses. Over most of the period range the losses measured for optimally loaded system lie between those for the two extreme cases. This is not an unexpected result and suggests that the system losses increase monotonically as the amplitude of motion of the device increases. At the 'resonant' period, however, the matched device losses are only about one-tenth of the fixed and free device losses which are both about 50%, which suggests that velocity mismatch in the normal direction contributes more to losses than do shear velocity gradients.

To see whether power was being lost or propagated in some preferred direction of rotation, the reciprocity of the system⁴

was measured by repeating our measurements with waves incident in the reverse direction, that is, on the rear of the device. We found that the same fraction of power $(C/A)^2$ was transmitted in either direction within experimental error over the whole range of wavelengths, and so the system was reciprocal.

For those losses caused by boundary layer effects and turbulence, the fractional power loss will be dependent on the Reynold's number (Re). As λ is proportional to T^2 (ref. 3), Re is proportional to T^3 and the Reynold's numbers at full scale will be nearly 10,000 times greater than in our experiments, and these losses would be greatly reduced.

Experiments with our model have shown that good power conversion efficiencies (more than 50%) can be obtained over the range of wave periods (a 2:1 bandwidth) corresponding to that found in ocean waves. Further work is needed to establish in detail the response to complex wave fields with multiple frequencies from various directions, the effects of real sea conditions, the effect on performance of alternative loading systems and the precise way in which losses scale. It is, however, already clear that the rocking boom device is very promising as the basis of a wave power system. The practical effects that we have investigated, of losses and bandwidths, do not seem to represent serious limitations on performance.

This work was carried out at Marchwood Engineering Laboratories and at the University of Edinburgh, which provided experimental facilities and equipment.

D. T. SWIFT-HOOK
B. M. COUNT
I. GLENDENNING

Marchwood Engineering Laboratories,
Central Electricity Generating Board,
Marchwood,
Southampton SO4 4ZB

S. SALTER

Mechanical Engineering Department,
University of Edinburgh,
Edinburgh EH8 9YL, UK

Received December 16, 1974; revised February 28, 1975.

¹ Salter, S., *Nature*, **249**, 720-724 (1974).

² Draper, L., and Squire, E. M., *Trans. R. Instn. nav. Archit.*, **109**, 85-93 (1967).

³ Milne Thomson, L. M., *Theoretical Hydrodynamics*, 388-439 (Macmillan, London, 1962).

⁴ Aroian, L. A., et. al., *Scientific Encyclopedia*, 1503 (Van Nostrand, New York, 1968).

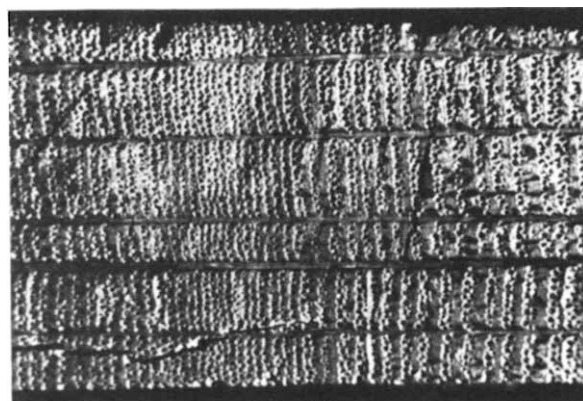
the earlywood of two of the annual rings are abnormally small (Fig. 1). This peculiarity has subsequently been observed in about 0.4% of the 24,000 rings measured on these panels and often, but not invariably, appears in the same annual ring on both ends of a board as well as on separate boards prepared from the same tree. I have also found examples on the boards of a late mediaeval cupboard (Fig. 1) made for an Oxford College⁵, and on timbers used for the Roman quay to the Thames at London, but none in the stems of 30 trees⁶ felled recently at an age of about 150 yr.

For the panels (almost all their ring-width charts have been matched and dated by reference curves) it soon became apparent that the effect was mainly confined to three specific years in the period 1433-55. At present this period is covered by 109 dated boards derived from 81 trees. Examples occur on 47 of these boards (derived from 36 trees) there being 36 occasions for the year 1437; 12 for 1443; and 21 for 1454. Six of the same boards also had earlywood vessels smaller than usual for one or more of the years 1438, 1439, 1444, 1445, and 1455, the total of such instances being ten. In the fourteenth century the abnormality appears for 1348 and 1390 (with eight and five occasions respectively); the remaining 20 examples are distributed sporadically between 1260 and 1540.

The presence of earlywood vessels has proved to be diagnostic for dating. They were observed in two rings separated by 17 yr on the portrait of Elizabeth Woodville in the Royal Collection at Windsor Castle. With the dates 1437 and 1454 thus suggested for the two rings, the 116-yr sequence matched well with signatures on one of our reference curves (Fig. 2). It is therefore dated with high reliability even though it is relatively short in length because of an imperfection on part of the edge.

Vessel development commences almost simultaneously throughout the stem cambium of a large oak by very complex processes⁶. When the buds flush in April-May, the vessels expand apparently under the stimulus of auxin and other compounds. K. A. Longman and K. R. Shephard have suggested (personal communication) that interference with cambial activity by a climatic factor, perhaps limited to only a few days and directly or indirectly related to flushing, might cause earlywood vessels to be abnormally small. A purely local cause can be dismissed as examples occurred in 1433 in woodlands near Oxford, near London and in the Low Countries. The effect in the period 1433-55 may

Fig. 1 Part of the edge, 1.8 cm wide, of an oak board, with 277 annual rings spanning 1209-1485, showing abnormally small earlywood vessels for 1437. This board and another form one of the 18 doors of a large cupboard made for Corpus Christi College, Oxford, in the period 1515-20, when the College was newly founded.



1437

Direction of growth →

Relation of abnormal earlywood in oaks to dendrochronology and climatology

TREE-RING investigations in Europe are largely based on the method initiated by Huber in 1938 at Tharandt (near Dresden)¹ by which the widths of the rings are measured and the resulting sequence charted and compared both visually and by computer. In temperate zones such as England and the Low Countries oak panels are ideal for the study of dendrochronology as they were prepared from the butt logs of elite trees of slow and even growth. A panel used as a support for a painting has the added advantage that its edges are accessible². Since 1971 I have worked on 115 of those used by portrait painters in South-east England or Flanders between 1450 and 1620 (refs 3, 4). The boards (there are more than one on some panels) have an average of 200 rings of mean width 1.5 mm.

At an early stage a curious feature was noticed on the panel of Holbein's portrait of Robert Cheseman; all the vessels in

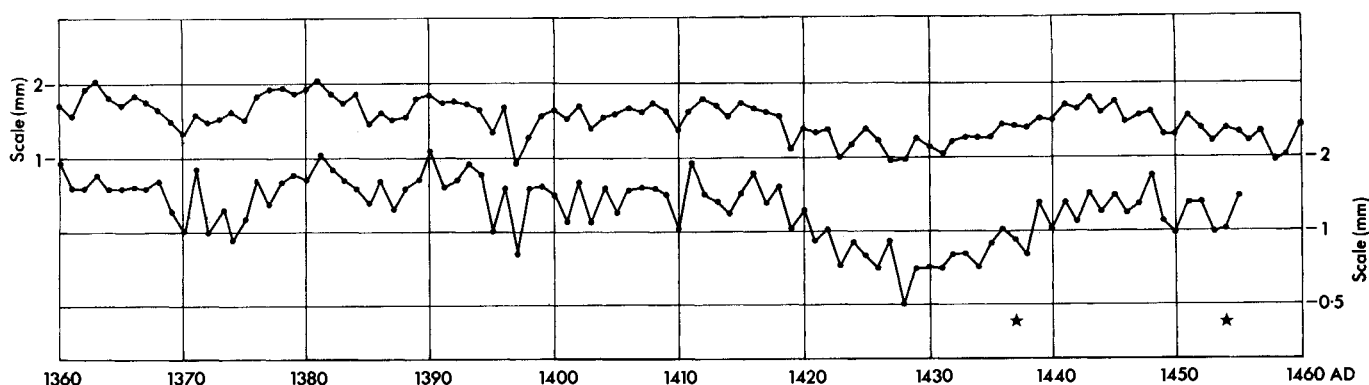


Fig. 2 A 100-yr section of the ring-width chart (semi-log scale) for the oak panel support of the portrait of Elizabeth Woodville. It agrees closely with the signatures on the reference curve. The stars mark the two years with abnormally small earlywood vessels. Upper, Reference curve (18 trees, left hand scale); lower, Elizabeth Woodville (right hand scale).

well be linked with the exceptionally severe winters and cold springs⁷⁻⁹ in central and western Europe between 1428 and 1450, but more specific factors must have been involved as it did not coincide with each. Whatever the cause, there is no indication that the width of the subsequent latewood was significantly altered.

Both Flohn¹⁰ and Lamb^{8,9} associate the cold winters and springs of the 1430s and 1440s with a long period of blocking conditions which lessened the frequency of the westerly winds over Britain and much of north-western Europe. Since the annual rings of numerous oaks that have remained buried from about 6000 BC are now being measured at Stuttgart¹¹, Cologne¹² and Belfast¹³, observations of abnormal earlywood may extend our knowledge of variations in Europe's climate in Flandrian times.

This research has been supported by the Leverhulme Trust Fund. It owes much to the permission graciously granted by Her Majesty The Queen to work on paintings at Windsor Castle, and to the co-operation of the Surveyor of the Queen's Pictures, the Director of the National Portrait Gallery, the Society of Antiquaries and owners of private collections. I thank the members of the Department of Forestry for help with the computer work.

J. M. FLETCHER

Research Laboratory for Archaeology
and The History of Art,
Oxford University,
6 Keble Road, Oxford, UK

Received February 6, 1975.

- ¹ Huber, B., *Handbuch der Mikroskopie in der Technik*, 5, 171-211 (1970).
- ² Bauch, J., and Eckstein, D., *Stud. Conserv.*, 15, 45-50 (1970).
- ³ Fletcher, J. M., Tapper, M. C., and Walker, F. S., *Archaeometry*, 16, 31-40 (1974).
- ⁴ Fletcher, J., *Burlington Mag.*, 116, 250-258 (1974).
- ⁵ Fletcher, J. M., in *The British Oak*, (edit. by Morris, M. G., and Perring, F. H.), 80-97, (Classey, Faringdon, 1974).
- ⁶ Longman, K. A., and Coutts, M. P., *ibid.*, 194-221 (1974).
- ⁷ Hennig, R., *Abh. preuss. met. Inst.*, 2, No. 4 (1902).
- ⁸ Lamb, H. H., *The Changing Climate*, 94-102 and 181-188 (Methuen, London, 1968).
- ⁹ Lamb, H. H., *Endeavour*, 33, 40-47 (1974).
- ¹⁰ Flohn, H., in *Die Schwankungen und Pendlungen des Klimas in Europa* (edit. by H. v. Rudloff), 81-90 (Viewegverlag, Braunschweig, 1967).
- ¹¹ Becker, B., *Jb. Akad. Wiss. Lit. Mainz*, 143-145 (1972).
- ¹² Schmidt, B., *Archäol. KorrespBl.*, 1, 155-158 (1973).
- ¹³ Pilcher, J. R., *Tree-Ring Bull.*, 33, 1-5 (1973).

Chlorophyll-*a* photovoltaic cells

ALTHOUGH the properties of an organic photovoltaic cell in which the photoactive material is microcrystalline chlorophyll-*a* (Chl-*a*). In a sandwich configuration having chromium and mercury as the electrodes, the Chl-*a* cell, (Cr|Chl-*a*|Hg), achieves a power conversion efficiency of the order of $10^{-2}\%$, which is among the highest ever reported for organic photovoltaic cells^{1,2}. In contrast to the usual methods for preparing thin films (such as vapour deposition), a thin microcrystalline Chl-*a* film is prepared on a Cr electrode surface by the simple method of electrodeposition³. Such a film is fairly uniform and is uniquely efficient in the photocharge generation. The microcrystalline form of Chl-*a* differs from the aggregated form of monomer Chl-*a* in that it has an ordered structure and has a strong absorption band in the far red, peaking at 740-745 nm. A water-Chl-*a* adduct structure has been proposed for this type of Chl-*a* (ref. 4).

The fabrication of the metal-Chl-*a*-metal sandwich cells (including the (Cr|Chl-*a*|Hg) cell has been described elsewhere⁵. The electrodeposited Chl-*a* films used in most of the Chl-*a* photovoltaic cells are about 1,500 Å thick as measured by interferometry. The corresponding optical density of the Chl-*a* film at 745 nm is about 1.5. Monochromatic illumination is through the semitransparent Cr electrode ($\sim 30\%$ transmission). The intensity of the light incident on the Cr electrode is about 10^{-3} W cm⁻² at 745 nm.

The electrical conduction of the (Cr|Chl-*a*|Hg) cell in the dark exhibits a strongly non-ohmic or rectifying behaviour. The cell is found to be forward biased when a negative voltage is applied to the Cr electrode. Figure 1 shows a semilogarithmic plot of the dark current against applied voltage, the *i*-*V* characteristics, of a (Cr|Chl-*a*|Hg) cell. The rectification ratio (forward bias current/reverse bias current) is as high as 10^3 . The photoconduction behaviour is shown in Fig. 2. Curve (a) represents the same dark *i*-*V* characteristics as in Fig. 1 but plotted on a linear scale. Curves (b) and (c) represent the photocurrent as a function of applied voltage (photo *i*-*V* characteristics). These two curves are obtained when the cell is illuminated by 745 nm light having intensities of 7.6×10^{-4} and 2.7×10^{-3} W cm⁻² respectively. The insert in Fig. 2 represents the photovoltaic *i*-*V* characteristics. Here there is no applied voltage. Instead, photovoltage alone is present and

the cell current is varied by changing the external load resistance, R_L , in series with the cell from 10^4 to $10^{11}\Omega$. In this case the cell is under constant illumination by 745 nm light at a level of $7.6 \times 10^{-4} \text{ W cm}^{-2}$. Note how the shape of this purely photovoltaic i - V curve is very similar to the lower right quadrant portion of the i - V curve (1). The area of the largest rectangle enclosed by such an i - V curve represents the maximum power obtained from the cell at this light level. This maximum power of $1 \times 10^{-8} \text{ W}$ is found to couple through a R_L of $3 \times 10^6 \Omega$. With an incident power of $6 \times 10^{-5} \text{ W}$ at 745 nm on the Hg contact area (0.08 cm^2), the power conversion efficiency is about $1.6 \times 10^{-2}\%$. If correction for the light absorbed by the Cr layer is made, the efficiency is more nearly $5 \times 10^{-2}\%$. The open circuit voltage, V_{oc} , is about 0.32 V for this cell, and is nearly independent of the light intensity at this light level. On the other hand, the short circuit current, i_{sc} , shows a linear dependence on the incident light. The ratio i_{sc}/I , where I is the photon flux upon the Chl-*a* (that is, corrected for absorption by Cr) is 0.007. This represents the minimum quantum yield of charge generation in Chl-*a* when the cell is operating in the photovoltaic mode.

It must be noted the cell described above is one of the best found in this study. The performance can vary by as much as an order of magnitude from the poor to good cells. Table 1 summarises the data obtained from ten (Cr|Chl-*a*|Hg) cells. The average power conversion efficiency is about $5 \times 10^{-3}\%$. These cells are very stable and retain their characteristics for at least a month (in the dark) without obvious deterioration. Slow degradation under more intense light ($> 10^{-2} \text{ W cm}^{-2}$ at 745 nm) is seen. At the light level of $10^{-3} \text{ W cm}^{-2}$, a good cell can sustain a constant i_{sc} of the order of 0.1 μA over a period of hours.

Temperature dependence studies have also been performed on some of the cells. Between room temperature and $\sim -40^\circ\text{C}$ the photovoltaic i_{sc} exhibits a very small dependence on

Fig. 1 Dark i - V characteristics of a (Cr|Chl-*a*|Hg) cell. Note the forward and the reverse biased currents have opposite signs. $\circ$, Forward bias; $\triangle$, reverse bias.

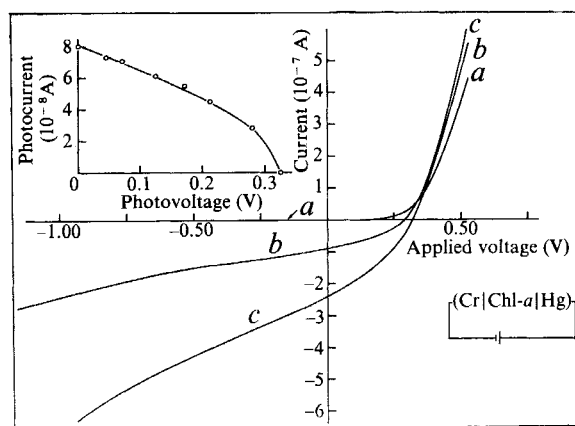
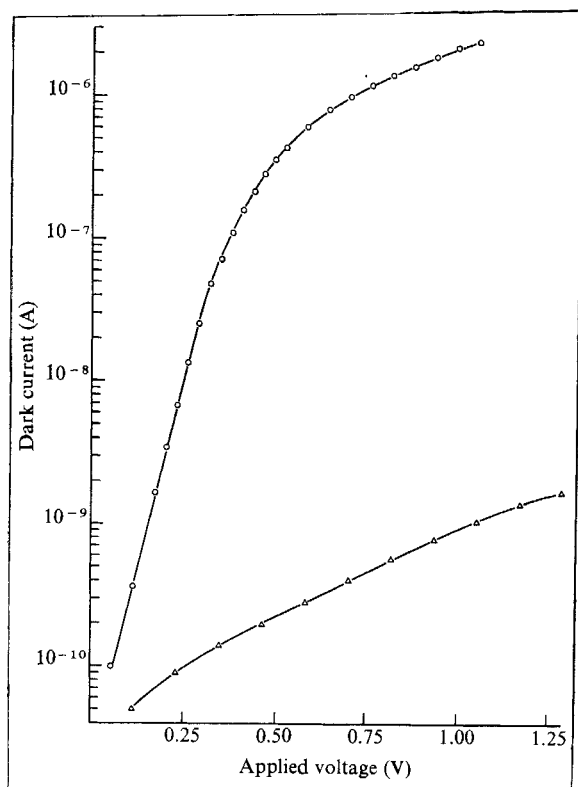


Fig. 2 i - V characteristics. (a), Cell unilluminated; (b) and (c), cell illuminated by 745 nm light of intensities 7.6×10^{-4} and $2.7 \times 10^{-3} \text{ W cm}^{-2}$ respectively. The insert is the photo i - V characteristics of the (Cr|Chl-*a*|Hg) obtained by varying the load resistance while the cell is constantly illuminated by 745 nm light.

temperature, whereas the dependence of the dark forward-bias current is considerably stronger. The Arrhenius plots give an activation energy of less than 0.05 eV for the photovoltaic i_{sc} and for the dark forward bias current values of 0.2 to 0.5 eV are found, depending on the sample.

The remarkable properties of the (Cr|Chl-*a*|Hg) photovoltaic cell may be explained in terms of the existence of a contact potential barrier (or Schottky barrier) at the Cr|Chl-*a* contact. Chl-*a* has been shown to exhibit p-type semiconduction⁶. It is generally true that a potential barrier often exists at the contact between a p-type semiconductor and a metal of low work function⁷. Thus, in the (Cr|Chl-*a*|Hg) cell the dominant Schottky barrier is at the Cr|Chl-*a* boundary, while the Chl-*a*|Hg contact possibly can be ohmic, or only slightly blocking. The asymmetric potential distribution provides the basis for the dark current rectification and the photovoltaic characteristics. The forward bias dark current in Fig. 1 is seen to follow a modified Shockley equation⁸ for a rectifying contact: $i = i_0[\exp((eV - iR_s)/nkT) - 1]$ where $i_0 = 2 \times 10^{-11} \text{ A}$ and $n = 1.6$. The iR_s term represents the potential drop across the series resistance, R_s , in the (Cr|Chl-*a*|Hg) photovoltaic cell. Its value is found to be $1.2 \times 10^5 \Omega$ and is orders of magnitude larger than that found in inorganic photovoltaic cells such as the silicon solar cell⁹.

The action spectrum of i_{sc} of the (Cr|Chl-*a*|Hg) cell is seen to match closely the absorption spectrum of the Chl-*a* film⁵. The optical thickness of the Chl-*a* film at 745 nm is such that were the photoactive domain located at the back contact, Chl-*a*|Hg, a significant "inner filter" effect (a quenching of the photoelectric response) would be seen. More detailed analysis⁵ has shown that the width of the photoactive region in the Chl-*a* film adjacent to the Cr electrode is a few hundred angstroms. Light absorbed by the bulk of the Chl-*a* beyond this region is presumably not very effective in producing free carriers. The minimum quantum yield of charge generation in the Chl-*a* film which is 0.007 with no bias, is found to increase under reverse bias (see lower left quadrant of Fig. 2). The reverse bias, which increases the barrier dimensions, evidently serves to partially overcome otherwise inefficient steps involving primary charge generation or subsequent recombination events, or both¹⁰. Meilanov *et al.*⁶ have studied (Al|Chl-*a* (non-crystalline) Al) cells at thicknesses far exceeding ours and at much higher applied voltage. Studies in the photovoltaic mode were not presented. A voltage dependent yield as high as 0.1 was observed however.

We have also attempted to study the dependence of the photovoltaic effects of the Chl-*a* sandwich cells on the electrode materials⁸. A correlation between the photovoltaic activity and the work function of the metals is observed, which further indicates that the Schottky barrier is directly responsible for the photoactivity. For instance, in a (Cr|Chl-*a*|Ga) cell, where Ga has a lower work function than Cr, the dominant Schottky barrier is established at the back, Chl-*a*|Ga, contact. The observed photovoltaic activity is dramatically different. The sign of the i_{sc} is now in a direction opposite to that seen in the (Cr|Chl-*a*|Hg) cell. Furthermore, the i_{sc} spectral response shows maxima in the spectral regions where the Chl-*a* film only absorbs slightly, that is, an "inner filter" effect is observed.

Table 1 Photovoltaic characteristics of ten (Cr|Chl-*a*|Hg) cells

Cell	V_{oc} (V)	i_{sc} (in 10^{-8} A)	P_{max} (in 10^{-8} W)	P_{max}/P_{in} (in $10^{-2}\%$)
1	0.32	8.0	1.00	1.6
2	0.31	6.0	1.08	1.0
3	0.18	3.9	0.43	0.43
4	0.19	7.3	0.47	0.68
5	0.18	5.0	0.30	0.50
6	0.21	5.0	0.38	0.63
7	0.18	8.0	0.48	0.80
8	0.30	7.5	0.80	1.40
9	0.20	6.0	0.50	0.83
10	0.25	2.5	0.40	0.67

V_{oc} is the open circuit voltage, i_{sc} is the short circuit current at the 745 nm light intensity of $7.6 \times 10^{-4} \text{ W cm}^{-2}$. P_{in} is the incident radiative power on the cell (no correction is made for the power absorbed by the Cr). P_{max}/P_{in} is the photovoltaic power conversion ratio.

The Schottky barrier model for the system predicts an activation energy for a forward bias current which consists of a voltage dependent term, $-eV/n$, and the barrier height (contained exponentially in i_0) at the Cr|Chl-*a* contact⁸. Accordingly, after correcting the observed activation energy for the forward bias voltage, barrier heights on the order of 0.4–0.7 eV are obtained, depending on the sample. Measurements of activation energies on a single sample at different bias voltages, or with cells having different electrode materials should test further the contact barrier model for the Chl-*a* system. The activation energy seen for the photovoltaic current seems to be exceptionally low for organic systems. Evidently, the steps leading to charge generation and transport following light absorption by Chl-*a* involve at most very weakly activated processes.

A.C.A. thanks Drs H. J. Hovel and J. M. Woodall of the Thomas J. Watson Research Center of IBM for their helpful criticism and for discussions on the general topic of photovoltaic cells. We thank the National Institutes of Health for support.

C. W. TANG
A. C. ALBRECHT

Department of Chemistry,
Cornell University,
Ithaca, New York 14850

Received December 27, 1974; revised February 25, 1975.

- Lyons, L. E., and Newman, O. M. G., *Aust. J. Chem.*, **24**, 13 (1971).
- Ghosh, A. K., Morel, D. L., Feng, T., Shaw, R. S., and Rowe, C. A., *J. appl. Phys.*, **45**, 230 (1974).
- Tang, C. W., and Albrecht, A. C., *Mol. Cryst. Liq. Cryst.*, **25**, 53 (1974).
- Ballschmitter, K., and Katz, J. J., *Biochim. biophys. Acta*, **256**, 30 (1972).
- Tang, C. W., and Albrecht, A. C., *J. Chem. Phys.* (in the press).
- Terenin, A., Putseiko, E., and Akimov, I., *Disc. Faraday Soc.*, **27**, 83 (1959).
- Bube, R. H., in *Photoconductivity of Solids*, 114 (Wiley Interscience, New York 1960).
- Sze, S. M., in *Physics of Semiconductor Devices*, ch 8 (Wiley Interscience, New York, 1969).
- Anderson, W. A., Delahoy, A. E., and Milano, R. A., *J. appl. Phys.*, **45**, 3913 (1974).
- Meilanov, I. S., Benderskii, V. A., and Blyumentfel'd, L. A., *Biofizika*, **15**, 822 (1970).

Can linguistic competence be dissociated from natural language functions?

THE preservation of non-verbal intellectual functions in spite of severe disruption of natural language has been well documented by studies of aphasia¹. But are the cognitive functions specific to normal verbal communication also preserved in the absence of language? In addition to its theoretical significance, this question has practical importance with respect to both infra-human primates and severe aphasics². These groups cannot use natural language because of the destruction or apparent absence of crucial cortical zones of the brain and effective communication may depend on their mastering an alternative symbol system which captures relevant cognitive properties of natural language. A crucial test of the hypothesised dissociation between natural language and its cognitive prerequisites is whether aphasic subjects can use arbitrarily designed symbols to represent elements of experience (events, properties, actions and so on); encode meaningful relationships in terms of the configurational properties, or syntax, of the symbol sequence; and have the capacity to encode such relationships in alternative syntactic forms^{3,4}.

We have verified the preservation of these cognitive concomitants of natural language in a severely aphasic patient. W.S. is a 21-yr-old right-handed male who was evaluated for language after surgical removal of a large, acute subdural haematoma of the left temporo-parietal lobe following severe head trauma. Ten weeks after surgery, at the time of admission to our unit, he had mild weakness in his right seventh cranial nerve and probable loss of smell sensation on the left; the remainder of his neurological and physical examination was normal. At this time he was observed to have very poor comprehension of spoken and written language. His speech was markedly paraphasic and devoid of information. On the Boston Diagnostic Aphasia Examination⁵, he scored near zero on subtests of auditory comprehension, naming, reading comprehension and writing. But he seemed to be extremely well oriented and alert, and when his intelligence was tested he executed the block design, picture arrangements and object assembly subtests of the Wechsler Adult Intelligence Scale at a superior level (scale scores of 12).

W.S. entered our research-therapy programme called VIC (Visual Communication) in which messages are formed by laying out—from left to right—index cards on which arbitrary or ideographic symbols stand for nouns and verbs, grammatical morphemes, sentence modality and tense markers, and meta-linguistic signals. The patient observes VIC communicators 'converse' with one another and then is gradually drawn into the conversation himself. Within two 30-min sessions W.S. had mastered the basics of the system; he demonstrated knowledge of the meaning of VIC symbols and the rules of the syntax governing VIC communication. Specifically, he could follow commands, answer simple questions, and describe events, with the total number of possible transactions running into the hundreds. After 14 additional 30-min sessions of VIC, W.S. could transmit and receive information involving direct and indirect objects, antonymic contrasts of verbs and of prepositions, judgments on the truth value of statements, and shifts in sentence modality, for example, from the interrogative to the declarative. More practically, W.S. was able to use the cards to express certain feelings and ideas; clearly VIC served as an alternative for his vitiated natural language capacity.

Here is a sample annotated exchange, translated into English. Word sequences designated by a single ideograph are connected by hyphens.

(1) Command involving a reordering of the element in the surface form of the utterance: Therapist (T): ! W.S. please-put spoon or fork to-the-right-of-the pencil. W.S. executes act with fork.

(2) Answering question requiring multiple specification of nouns and expansion of constituent: T gives pencil and razor to Y. T: ? Who give(s)-to what (to) whom. W.S.: T give(s)-to pencil and razor (to) Y.

(3) Appropriate response when confronted with a novel surface form: T picks up a cookie. T: ? What T pick-up [rather than normal form: ? T pick-up what]. W.S.: T pick(s) up cookie.

(4) Spontaneous utterance in VIC: T takes out pack of cigarettes. W.S.: W.S. want(s) cigarette (a few seconds elapse) and matches.

To establish that this success was not the result of a mapping of VIC on to residual or recovering natural language functions, W.S. was given an extensive test of spoken and written English, with all items equivalent to VIC messages. He performed without error on items in VIC, but scored only 12.5% correct in English.

The design of VIC suffices to demonstrate important conceptual operations entailed in ordinary language. First, W.S. seems to understand and use the symbols in terms of the normal categories of perceptual experience (for example, actor- action- object and indirect object of the action). Second, W.S. seems to understand the meaning relations among the categories in terms of a small number of configurations and to deal appropriately with novel messages; both of these capacities suggest that he can understand VIC sequences in terms of the relations among the component elements. Third, W.S. is not bound by referentially constrained situations in his use of VIC. Finally, W.S. seems to exhibit the beginnings of productive use of VIC, by virtue of his ability to expand on constituents that request information.

The case of W.S. shows that certain cognitive capacities entailed in natural language can be preserved in its absence. This patient was unique among VIC communicators in the speed and facility with which he mastered the system. The fact that five out of eight patients tested have mastered the basic components of the system within 6 weeks, however, and have performed at a level superior to their performance with English, indicates at least some dissociation among the two functions in severely aphasic patients.

The performance of W.S. can be compared usefully with those of Sarah⁶, Washoe⁷ and Lana⁸, primates used in communication studies; however, his achievement differs not only in its overall level of proficiency, but also in the apparent fact that Sarah has not used her symbol system for spontaneous communication while Washoe has not used syntax consistently. The skill of W.S. at mapping his knowledge of the environment on to a symbol system without the apparent aid of a mediating system is most reminiscent of that found in young children, and, perhaps, in Lana. Possibly our patient is in the paradoxical position of retaining cortical capacity for communication using symbols while having lost, at least temporarily, the ability to handle natural language.

This research was supported in part by the National Institute of Neurological Disease and Stroke.

ERROL BAKER
THOMASIN BERRY
HOWARD GARDNER
EDGAR ZURIF
LYNN DAVIS
AMY VEROFF

*Aphasia Research Center,
Boston University School of Medicine and
Psychology Service,
Boston Veterans Administration Hospital,
Boston, Massachusetts 02130*

Received October 18, 1974; revised February 14, 1975.

¹ Zangwill, O., in *Disorders of Language* (edit. by DeReuck, A., and O'Connor, M.), 261-274 (Churchill, London, 1964).

² Velletri-Glass, A., Gazzaniga, M., and Premack, D., *Neuropsychologia*, **11**, 95-104 (1973).

³ Brown, R., *A First Language* (Harvard University Press, Cambridge, 1973).

⁴ Fodor, J., Bever, T., and Garrett, M., *The Psychology of Language* (McGraw-Hill, New York, 1974).

⁵ Goodglass, H., and Kaplan, E., *The Assessment of Aphasia and Related Disorders* (Lea and Febiger, Philadelphia, 1972).

⁶ Premack, D., *Science*, **172**, 808-822 (1971).

⁷ Gardner, R. A., and Gardner, B., *Science*, **165**, 664-672 (1969).

⁸ Rumbaugh, D. M., Gill, T. V., and von Glasersfeld, E. C., *Science*, **182**, 731-733 (1973).

Intensification of the alcohol withdrawal syndrome by repeated brain stimulation

SINCE the discovery that electrical stimulation of discrete sites of the brain can influence behaviour, there have been numerous reports of its application¹. Such procedures, however, may lead to kindling²; stimulation of some sites at currents initially too low to produce a behavioural response may lead to a gradual development and then intensification of seizures evoked by the stimulation³. Thus when the brain is repeatedly stimulated for therapeutic purposes, seizures may be elicited unless procedures not conducive to kindling are used². Two questions arise: can kindling leave an organism more susceptible to seizures produced by agents other than local brain stimulation, and are elicited convulsions necessary for this change in susceptibility? Our findings indicate that whether or not overt convulsions are produced by brain stimulation, repeated electrical stimulation of the brain potentiates the effects of subsequent alcohol withdrawal.

To answer the first question we studied the interaction of kindling and alcohol. When large quantities of ethanol have been metabolised an organism is in a state of heightened seizure susceptibility⁴. In alcoholics this withdrawal syndrome is often characterised by tremors and other mild epileptic symptoms, and *grand mal* convulsions and death can ensue. Even in organisms that display no obvious symptoms, there is an increased susceptibility to seizures induced by other agents after withdrawal⁴. Hence, if kindling affects general seizure susceptibility, this should be reflected in an intensification of the alcohol withdrawal syndrome.

Twenty-seven, male, black-hooded rats (Canadian Breeding Farms, La Prairie, Quebec) were implanted with unilateral, bipolar electrodes aimed at the centre of the amygdaloid complex. After 1 week of recovery, 12 randomly selected rats were kindled with 45 brain stimulations (1 s, 400 μ A, 60 Hz) administered three times per day, 5 d per week. The animals were kindled successfully in that each eventually displayed at least one bilateral seizure involving clonic activity of the head, mouth and fore-limbs accompanied by rearing and loss of equilibrium. The remaining implanted animals served as unstimulated controls.

Three days after the last stimulation, rats in both groups received 45 intubations of a 20% volumetrically prepared alcohol solution (1,000 mg kg⁻¹) as before⁵. Intubations were at 8-h intervals and intoxication was tested 1 h after each intubation. An animal was considered intoxicated when it exhibited two or more of the following symptoms: (1) unconsciousness before handling, (2) inability to manoeuvre on a vertical screen or (3) ataxia. If there were no signs of intoxication, subsequent doses were increased by 200 mg kg⁻¹ until intoxication was produced. All animals developed a tolerance to the alcohol; the mean difference between the first and last intoxicating dose was 870 mg kg⁻¹ and every animal showed an increase of at least 200 mg kg⁻¹ ($\chi^2 = 20.02$; $P < 0.001$). Before withdrawal the body weights (mean = 342 g) and effective intoxicating doses (mean = 2,018 mg kg⁻¹) were similar for stimulated and unstimulated animals.

Commencing 9 h after the last intubation each animal was placed under formal observation for 2 min every 3 h by an experimenter, unaware of the animal's experimental history, who recorded the presence or absence of the following seven withdrawal symptoms: rhythmic activity of the mouth, facial tremors, rhythmic eye or ear twitching, myoclonic body jerks, tail tonus, rhythmic head nodding and hyperreactivity to handling. Since there were six observation sessions, the maxi-

imum score an animal could receive for each symptom was 6, with an overall maximum of 42 for all symptoms combined.

The withdrawal syndrome observed in kindled animals was much more severe than that of the controls in terms of each symptom. To make an overall statistical comparison each animal was given a score for each symptom that was a percentage of the mean score obtained for the unstimulated control animals for that symptom. Thus, the mean of the control animals for each symptom was 100% and the control mean for all seven symptoms was also 100%. In contrast, the mean of these overall scores for the kindled animals was 249.8% ($U = 16$; $N_1/N_2 = 11/110$ $P < 0.005$, one-tailed). The incidence of seizures characterised by rhythmic head nodding, synchronous jaw movements and facial spasms over the six observation periods was also significantly greater in kindled animals (mean = 3.5) than controls (mean = 1.2) ($U = 18.5$; $N_1/N_2 = 11/11$; $P < 0.005$, one-tailed).

Repeated brain stimulation not only leads to seizures but also to a permanent decline of the afterdischarge (AD) threshold⁶. Kindling occurs only when the stimulation levels are above the AD threshold, but this threshold can be reduced even when stimulation has no electrographic or behavioural effects⁶. Thus even stimulation with no obvious effects can produce a lasting change in brain function.

To investigate whether such stimulation can intensify subsequent alcohol withdrawal, or whether elicited convulsions are necessary 11 animals were implanted with electrodes as before and an initial AD threshold was determined for each. Each animal was stimulated for 1 s with 25 μ A, then, if no AD was elicited, with 50, then 75 and so on at increments of 25 μ A every 6 h until an AD was produced. The intensity that evoked an AD was considered the threshold for that animal. Each animal was stimulated until only one AD was produced and then handled without stimulation until all thresholds had been determined. Subjects were then divided into two groups on the basis of their thresholds. One group ($n = 6$; mean = 131.2 μ A) was placed on a stimulation regimen similar to the schedule of the first experiment, with an initial stimulation intensity for each animal 15% below the threshold for that animal. Whenever an AD was produced, the intensity of subsequent stimulations was reduced by another 15%. In this way most stimulations were kept below the AD threshold. Thus, although the average threshold drop for the stimulated groups was 62.2%, no animals developed full motor seizures, and only one displayed mild behavioural symptoms in response to stimulation. The remaining animals ($n = 5$; mean = 134.3 μ A), served as unstimulated controls. Two days after the last stimulation all animals received alcohol intubations and underwent withdrawal as in the first experiment.

The raw scores for each withdrawal symptom were again converted to a percentage of the control mean for respective symptoms. As in the first experiment each symptom was more frequent in stimulated animals than in controls and the overall means (100.0% compared with 197.1%) differed significantly ($U = 3$; $N_1/N_2 = 5/6$; $P < 0.02$).

Our data show that repeated brain stimulation not only increases susceptibility to seizures produced by subsequent stimulations, but also renders the organisms more susceptible to other convulsive agents. Intensification of withdrawal symptoms by stimulation above or below the AD threshold suggests that the effect does not depend exclusively on the production of overt seizures, and that the reduction of the threshold increases the severity of withdrawal.

If brain stimulations are repeated for several months, spontaneous seizures occur, even after all stimulation ends^{7,8}. Thus, we think kindling reflects the development of an epileptic focus. Since the effects of various convulsive agents interact with epileptic foci, alcohol is probably not the only agent whose withdrawal symptoms are potentiated by prior kindling. More importantly, as ADs have been produced repeatedly in humans^{9,10} and as clinical evidence exists of kindling-like phenomena¹⁰, it may be necessary to re-evaluate some clinical practices, with regard to both possible kindling in brain stimulation treatments,

and the subsequent administration of convulsive drugs. The important consideration in such a re-evaluation is that even when the current intensities are too low to produce any electrographic or behavioural effects, enduring changes in brain function may be produced.

JOHN P. J. PINEL
P. H. VAN OOT
R. F. MUCHA

Department of Psychology,
University of British Columbia,
Vancouver, British Columbia,
Canada U6T 1W5

Received December 5, 1974; revised January 31, 1975.

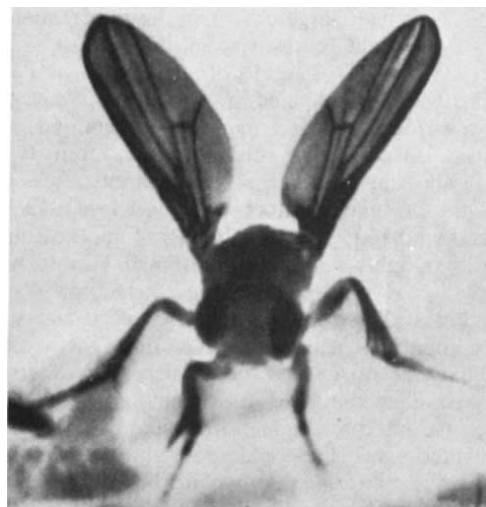
- ¹ Valenstein, E. S., *Brain Control* (Wiley, New York, 1973).
- ² Goddard, G. V., in *Neuroelectric Research* (Thomas, Springfield, Illinois, 1971).
- ³ Goddard, G. V., McIntyre, D. C., and Leech, C., *Expl Neurol.*, **25**, 295 (1969).
- ⁴ McQuarrie, D. G., and Fingl, E., *J. Pharmac. exp. Ther.*, **124**, 264 (1958).
- ⁵ Mucha, R. F., Pinel, J. P. J., and Van Oot, P. H., *Pharmac. Biochem. Behav.* (in the press).
- ⁶ Racine, R., *Electroenceph. clin. Neurophysiol.*, **32**, 269 (1972).
- ⁷ Wada, J. A., Sato, M., and Corcoran, M. E., *Epilepsia*, **15**, 465 (1974).
- ⁸ Pinel, J. P. J., Mucha, R. F., and Phillips, A. G., *Physiol. Psychol.* (in the press).
- ⁹ Sem-Jacobsen, C. W., *Depth-electrographic stimulation of the Human Brain and Behavior* (Thomas, Springfield, Illinois, 1968).
- ¹⁰ Heath, R. G., Monroe, R. R., and Mickel, W. A., *Am. J. Psychiat.*, **111**, 862 (1955).

Aggression and mating success in *Drosophila melanogaster*

THE value of *Drosophila melanogaster* as an experimental animal for the study of the genetics of behaviour increases as more specific behaviours are shown to exist in this species. Although aggressive behaviour has been reported for *D. subobscura*¹ and some Hawaiian² species, and has been briefly mentioned for many other species³, it has not yet been described for *D. melanogaster*. We have repeatedly observed behaviours which we interpreted as aggressive and here, report an experiment designed to investigate aggression and its relationship to mating success—an important component of fitness.

Flies from a mass-bred stock (Haren) which has been in the laboratory since July, 1971 were used in this experiment. To identify individuals, 12-h-old males were marked on the dorsal surface of the thorax with dots of acrylic paint while under light etherisation. They were kept singly in vials containing standard *Drosophila* medium until they were 3 d old. At this time, six males were placed in a cylindrical Perspex arena (7 cm diameter, 4 cm high) containing a small dish (2.5 cm diameter) with *Drosophila* medium. After an 18-h settling period, flies were observed with a binocular microscope. All interactions occurring on the food surface and their outcomes were recorded over a period of 3 h. In addition the

Fig. 1 A charging male of *D. melanogaster*: wing threat, wings spread, raised and twisted.



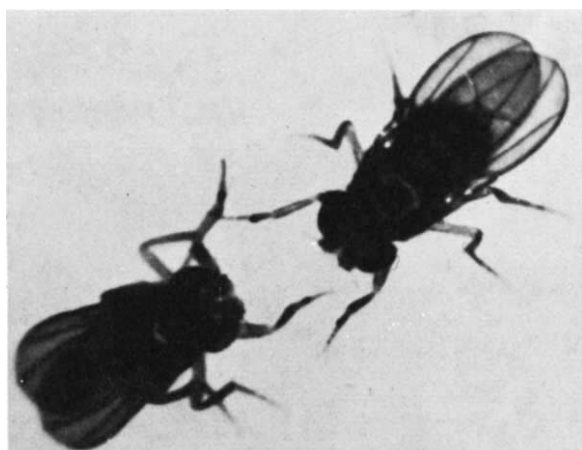


Fig. 2 Two males boxing.

position of the individuals in relation to the food surface was noted every 5 min. We then started to introduce 3-d-old virgin females at intervals of approximately 50 min, recording which males were successful in copulation. At the end of each day the females were removed.

The interactions observed were primarily aggressive or sexual. We found three frequently occurring aggressive behaviour patterns: wing threat (Fig. 1) which is often directed to other males just before a very quick charge, in which the aggressor usually rises on his hindlegs shortly before impact is made, and boxing (Fig. 2) which comprises several variations of very vigorous slashing and tapping with the front legs, often while both males rise on their hindlegs.

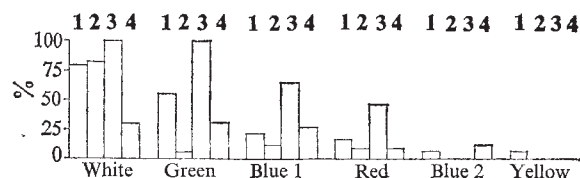


Fig. 3 Activities of each male, expressed as percentages. (1) Time spent on the food, 100% = total time of observation; (2) attacks initiated, 100% = total number of attacks made by all flies; (3) fights won, 100% = total of a given animal's fights; (4) copulations, 100% = total number of copulations by all males = 19.

The six males showed large differences in the frequency with which they stayed on the food surface, with which they attacked, were attacked, won and lost bouts of aggression and copulated (Fig. 3). The winner is defined as the animal which remains on the food surface after an aggressive encounter. Clearly, 'white' stayed on the food surface most often ($\chi^2=64.1$, 5 d.f., $P<0.005$), initiated most threats and attacks relative to the amount of time spent on the food ($\chi^2=31.0$, 3 d.f., $P<0.005$) and won 100% of his fights. Ranking the animals on the proportion of fights won gives the following result: 'white' = 'green' > 'blue 1' > 'red' > 'blue 2' = 'yellow'. This rank order is significantly correlated ($r=0.95$, $P<0.01$) with the number of copulations achieved by each male.

Drosophila males do court other males, and other investigators who have encountered the behaviours we have described may have interpreted them as sexual in nature. In well over 1,000 videotaped single pair courtships, however, none of the behaviour patterns that we define as aggressive has previously been observed. Milani¹ has described a behaviour

similar to our wing threat in *D. subobscura* and interpreted it as aggressive in nature. Brown⁴ dismisses this notion and prefers to call it counter-signalling. According to our observations, however, it cannot be a counter signal since a male engages in this behaviour before an interaction initiated by him. In *D. melanogaster*, wing flicking serves as a counter signal which is given in interactions involving physical contact between individuals of either sex.

The adaptive nature of aggression on a food source might be that males monopolise a food resource for reasons of energy requirement and/or because females will be attracted to it. Our results provide evidence that the more successful a male is in fights the fitter he is in terms of mating success.

This work was supported by scholarships from the National Research Council of Canada and the German Academic Exchange Service. We thank Professor A. Manning and Mr R. Wright for their helpful criticisms of the manuscript and Mr D. Cremer for his assistance with the photography.

MAURICE A. DOW
FLORIAN VON SCHILCHER

Department of Zoology,
University of Edinburgh,
West Mains Road,
Edinburgh EH9 3JT, UK

Received December 30, 1974; revised February 14, 1975.

¹ Milani, R., *Selected Scientific Papers, Istituto Superiore di Sanita*, 1, 213-224 (1956).

² Spieth, H. T., *Univ. Tex. Publ.*, 6615, 245-313 (1966).

³ Spieth, H. T., *Bull. Am. Mus. Nat. Hist.*, 99, 395-474 (1952).

⁴ Brown, R. G. B., *Behaviour*, 23, 61-106 (1964).

The function of phytochrome in plants growing in the natural environment

MANY aspects of plant development are subject to photocontrol by way of the chromoprotein photoreceptor phytochrome. Phytochrome exists in two forms, P_r , which absorbs maximally at 660 nm, and P_{fr} , which absorbs maximally at 725-735 nm^{1,2}. Absorption of light by either form results in phototransformation to the isomeric form. These properties make phytochrome a unique photoreceptor and raise important questions concerning the function of phytochrome in plants growing in the natural environment^{3,4}. In the physiological experiments which have been so successful in elucidating the details of the structure and properties of the molecule, and which are beginning to provide evidence on its cellular mode of action, aetiolated plants are usually used and are given abnormal irradiation treatments involving brief or prolonged exposure to radiation of restricted wavelengths. Such conditions do not occur in the natural environment and, therefore, such experiments provide no evidence on the role of phytochrome in plants growing in the field. Suggestions have been made that phytochrome may enable the plant to detect shading by other plants and thus induce the alteration of metabolism and development in an appropriate manner^{5,6}. These proposals point out that radiant energy below 700 nm is almost completely reflected or absorbed by vegetation, whilst that between 700-800 nm (that is, the far red) is largely transmitted⁷⁻¹⁰. The relative enhancement of the far red will presumably alter the photoequilibrium of $P_r \rightleftharpoons P_{fr}$ in plants growing under vegetation canopies, presenting a possible mechanism for the detection of shading. No systematic attempts have yet been made, however, to determine the extent of the spectral energy changes in the natural environment, to correlate them with effects on phytochrome photoequilibria and to assess the possible regulatory role of phytochrome under these conditions. In this report we present preliminary evidence along these lines which is consistent with the above hypothesis.

Measurements of the spectral energy distributions (SED) of natural radiation between 400 and 800 nm were made using a spectroradiometer (Gamma, San Diego) equipped with a

flexible fibre-optic light guide and a remote miniature cosine-corrected receptor head. The instrument scanned the wavelength range in 40 s and the receptor head could be placed in any position within and outside crop canopies without disturbing the vegetation. The scanned spectra were corrected for instrument response by calibration against standard lamps (National Physical Laboratory, Teddington, UK). Phytochrome photoequilibria were determined by exposing freshly prepared aetiolated bean (*Phaseolus vulgaris*) hypocotyl hook sections in a plastic cuvette to the various natural and artificial radiation sources. The cuvette was cooled to 0 °C in an ice bath and exposed for a sufficient period of time for photoequilibrium to be reached. The P_{fr}/P_{total} proportions were then determined in a modified Perkin Elmer 156 dual wavelength spectrophotometer using standard procedures^{11,12}. All P_{fr}/P_{total} data presented are the means of at least 12 determinations.

Table 1 Photoequilibrium dependence on $E_{660}:E_{730}$ ratio

	$E_{660}:E_{730}$	P_{fr}/P_{total}
Midday daylight	0.99–1.17	0.59–0.62
Wheat canopy	0.21	0.33
Sugar-beet canopy	0.033–0.045	0.06–0.10
Incandescent	0.71	0.55
Fluorescent	13.5	0.76
Red	20.1	0.80
Far red	0.002	0.04

The values for the wheat canopy are the means of measurements taken at ground level on June 27, 1974. For sugar-beet, a typical range of values are presented from measurements in the dense shade below one or more leaves on June 11, 1974. Red light was produced by filtering the light from eighteen 2 foot × 20 W red fluorescent tubes through two layers of No. 14 ruby Cinemoid (Rank Strand Electric, London). Far red light was produced by filtering the light from 40 150 W incandescent bulbs through two layers of No. 5A deep orange and two layers of No. 20 deep blue (primary) Cinemoid.

In a series of measurements made throughout the growing seasons of 1973 and 1974, only slight variation was observed in the SED of natural daylight; more was seen in daylight filtered through wheat and sugar-beet crop canopies. 'Typical' midday SED scans of natural daylight and canopy light (that is, daylight filtered through a vegetation canopy) are shown in Fig. 1. The very large relative enrichment of far red wavelengths in canopy light is apparent. When seeking to correlate changes in SED with phytochrome photoequilibria, it is useful to have a single parameter to describe the SED data. The simplest, though possibly not the best, is the ratio of absolute energy, in quantum terms, of the wavelengths of maximum absorption of P_r and P_{fr} , that is, $E_{660}:E_{730}$. It is possible to derive this value from the spectroradiometer scans, and we have been able to show that terrestrial plants normally exist under conditions in which the $E_{660}:E_{730}$ ratio ranges from about 1.2 in full sunlight, to about 0.05 in dense vegetation.

Using a variety of characterised natural and artificial radiation sources providing $E_{660}:E_{730}$ values from almost 0 to 20 we have

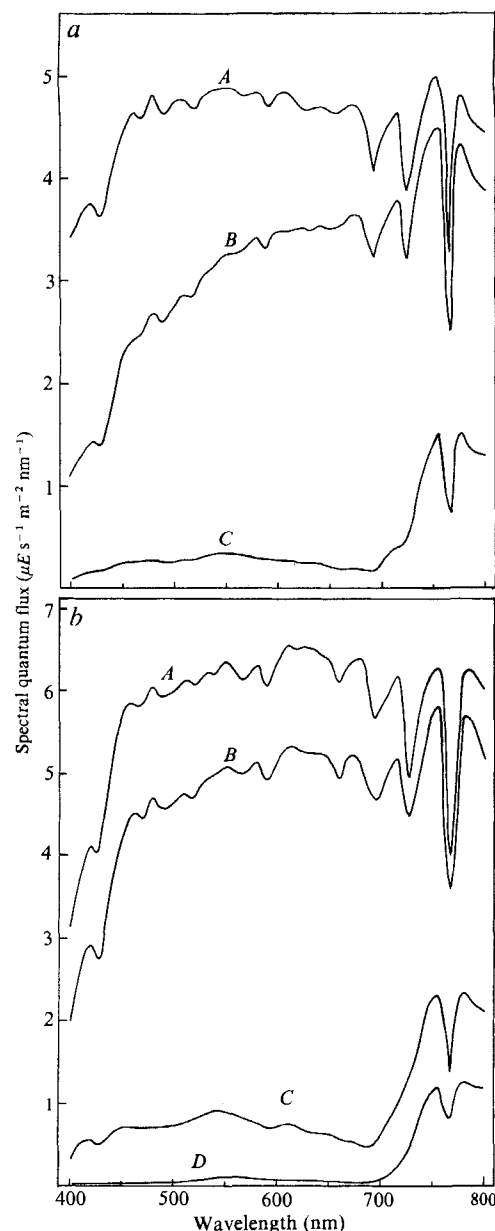


Fig. 1 SED of daylight above and below two crop canopies. *a*, Winter wheat canopy with a partially clear sky measured between 1000 and 1020 GMT on June 14, 1974: *A*, above canopy; *B*, ground level in sunfleck; *C*, ground level in shade. *b*, Sugar-beet canopy with a partially clear sky measured between 1320 and 1335 GMT on August 6, 1974: *A*, above; *B*, ground level between rows; *C*, and *D*, ground level within rows.

Table 2 Developmental changes in *T. maritimum* and *C. album* over 15 days at 25 ± 1 °C under fluorescent (low far red) and incandescent (high far red) light sources

	<i>T. maritimum</i>		<i>C. album</i>	
	Fluorescent	Incandescent	Fluorescent	Incandescent
Height (cm)	29.7 ± 1.2	59.4 ± 1.4	15.0 ± 0.9	28.4 ± 1.5
Internode length (cm)	0.84 ± 0.09	3.53 ± 0.18	—	—
Leaf dry weight (g)	0.483 ± 0.029	0.464 ± 0.024	0.338 ± 0.016	0.310 ± 0.016
Stem dry weight (g)	0.329 ± 0.017	0.583 ± 0.032	0.104 ± 0.006	0.197 ± 0.009
Leaf: stem dry weight ratio	1.47	0.80	3.25	1.57
Leaf area (cm²)	—	—	107.1 ± 1.2	78.5 ± 0.9
Chlorophyll <i>a</i> + <i>b</i> (mg per g fresh weight)	75.7 ± 1.9	67.0 ± 1.7	112.3 ± 1.8	93.8 ± 4.5

The light sources were adjusted to produce equal quantum fluxes in the 400–700 nm waveband. *T. maritimum* seedlings were grown in a glasshouse until 9 weeks old, then transferred to fluorescent lighting in a growth chamber for 1 week before starting the treatments which were carried out in 8-h days. *C. album* were collected from an open situation in the field, potted and grown under fluorescent lighting for 10 d. The plants were then divided equally between the fluorescent and incandescent sources; 16 h photoperiods were used.

attempted to correlate SED to phytochrome photoequilibria. The results are shown in Table 1 and illustrate that natural conditions ($E_{880}:E_{730}$ from 0.05 to 1.2) provide P_{fr}/P_{total} values ranging from near 0 to about 0.6. The theoretical maximum P_{fr}/P_{total} is 0.81, resulting from the overlapping absorption spectra of P_r and P_{fr} . A relatively small change in $E_{880}:E_{730}$ causes a substantial change in P_{fr}/P_{total} , indicating that phytochrome may be a highly sensitive detector of SED in the red and far-red regions.

Although the typical values of photoequilibria presented here follow a predictable relationship to actinic flux quality, it would be incorrect for several reasons to predict actual P_{fr} levels in plants grown in light from these data. First, differential attenuation of light by masking pigments will alter intracellular light regimes. Second, the molecular environment of phytochrome is an important factor in determining the photoequilibria, and this cannot be assumed to be identical in etiolated and light-grown tissues¹³. Third, a substantial proportion of phytochrome may be in the form of phototransformation intermediates under conditions of natural irradiation¹⁴. There are no methods presently available, however, which allow the determination of phytochrome photoequilibria in green tissue.

The crucial question is whether or not the changes in phytochrome photoequilibria which we suspect occur in the natural environment actually control development. To test this, various species are being grown for prolonged periods under artificial irradiation conditions which give equivalent rates of photosynthesis, but have widely different $E_{880}:E_{730}$ ratios. Preliminary data of the effects of a fluorescent source and an incandescent source on the development of two common arable weeds are presented in Table 2. The light sources provided equal quantum fluxes in the 400–700 nm photosynthetically active region, but established different phytochrome photoequilibria (fluorescent source, 0.76 P_{fr}/P_{total} , and incandescent source, 0.55).

The pattern of development under the two sources is clearly very different, with the source providing lower P_{fr}/P_{total} causing markedly increased internodal elongation and reduced leaf size. It should be borne in mind that the photoequilibria under the incandescent source was only marginally lower than that in normal summer daylight, whereas that under the fluorescent source was higher than values ever observed in nature. It will therefore be necessary to carry out the technically much more difficult exercise of constructing irradiation sources giving high photosynthetic rates but very low P_{fr} levels before definitive growth experiments can be carried out. Even so, the striking developmental effects of the small reduction of the P_{fr}/P_{total} from 0.76 to 0.56 are consistent with the view that phytochrome acts to perceive the changed quality of natural radiation resulting from shading by other plants. On the basis of this admittedly circumstantial evidence we support the working hypothesis that the function of phytochrome is to enable the plant to react appropriately to shading. This property would be of undoubted adaptive value and would ensure the persistence of phytochrome throughout evolution.

This work was supported by a research grant from the ARC and an equipment grant from the Royal Society. The advice of Professor J. L. Monteith, and the assistance of Miss J. Elliott are gratefully acknowledged.

M. G. HOLMES
H. SMITH

Department of Physiology and Environmental Studies,
University of Nottingham,
School of Agriculture,
Sutton Bonington,
Loughborough LE12 5RD, UK

Received January 27, 1975.

¹ Hillman, W. S., *A. Rev. Pl. Physiol.*, **18**, 301–324 (1967).

² Briggs, W. R., and Rice, H. V., *A. Rev. Pl. Physiol.*, **23**, 293–334 (1972).

³ Shropshire, W., *Solar Energy*, **15**, 99–105 (1973).

⁴ Smith, H., in *Seed Ecology* (edit. by Heydecker, W.), 219–231 (Butterworths, London, 1973).

⁵ Taylorson, R. B., and Borthwick, H. A., *Weed Sci.*, **17**, 48–51 (1969).

⁶ Cumming, B. G., *Can. J. Bot.*, **41**, 1211–1233 (1963).

⁷ Woolley, J. T., *Pl. Physiol.*, **Lancaster**, **47**, 656–662 (1971).

⁸ Kasperbauer, M. J., *Pl. Physiol.*, **Lancaster**, **47**, 775–778 (1971).

⁹ Stoutjesdijk, P. H., *Acta Bot. Neerl.*, **21**, 185–191 (1972).

¹⁰ Federer, C. A., and Tanner, C. B., *Ecology*, **47**, 555–560 (1966).

¹¹ Hillman, W. S., *Physiologia. Pl.*, **18**, 346–358 (1965).

¹² Kendrick, R. E., and Frankland, B., *Planta*, **82**, 317–320 (1968).

¹³ Mumford, F. E., and Jenner, E. L., *Biochemistry*, **10**, 90–101 (1971).

¹⁴ Kendrick, R. E., and Spruit, C. J. P., *Planta*, **107**, 341–350 (1972).

Male sterility in wheat plants deficient in copper

IN higher plants, copper deficiency affects the reproductive phase more than the vegetative. Yields of grain may be markedly reduced or nil without much effect on yield of vegetative parts^{1–3}. Failure to produce seed may be caused by lack of sufficient photosynthate production or translocation, or to the absence of fertilised embryos. Although soluble carbohydrates were low in copper-deficient wheat leaves, the evidence reported here points to the non-viability of pollen as the primary cause of failure to set grain. Possible mechanisms for the induction of male sterility by copper deficiency are proposed, and its potential for use in plant breeding is discussed.

Copper-deficient wheat plants developed small anthers, commonly less than half as long as those of normal plants and of about one-tenth the volume (Fig. 1). Pollen grains were fewer in number, small, and often dented like deflated footballs (Fig. 1). Copper-deficient pollen did not stain with iodine-potassium iodide solution (Table 1 and Fig. 1), indicating non-viability⁴.

Table 1 Effect of copper on pollen production and viability in wheat

Copper status of plants	Pollen grain numbers per anther	Stained pollen grains (%)
Copper-deficient	76	0
Copper-sufficient	3,400*	95

Values are the means for nine anthers.

*This value was not determined in this study but from counts of pollen grains in four anthers of healthy plants of the same cultivar in another study.

Cross pollination experiments (Table 2) confirmed the non-viability of copper-deficient pollen and also showed the viability of the ovule. With the exception of two grains in one head, no seed was set from copper-deficient pollen; in contrast, pollen from copper-sufficient plants set grain with both copper-sufficient and copper-deficient ovules. In copper-deficient ovules, normal pollen set grain in 30% of the florets. Most ovules may have been viable since pollen was applied only once, and fertile florets were found in all parts of the heads.

Time-of-application studies showed that grain set from deficient plants responded dramatically when copper was applied until 12 weeks after sowing, after which there was no response, except in later tillers. The critical period in copper-deficient plants was the early boot stage, which approximates to meiosis in microsporogenesis. Synchronised meiotic divisions in a large number of pollen mother cells⁵ may produce a localised demand for copper exceeding that which the deficient plant can supply. This hypothesis could be tested by studying

Table 2 Grains set by cross pollination between copper-deficient and copper-sufficient wheat plants

Cross		No. of grains set	Total no.	
♀	♂		Florets	Heads
CD	CD (selfed)	0	76	3
CS	CD	2	76	3
CD	CS	47	157	7
CS	CS (selfed)	86	86	3

The values for the selfed heads are typical of many heads produced during these studies.

CD, copper-deficient; CS, copper-sufficient.

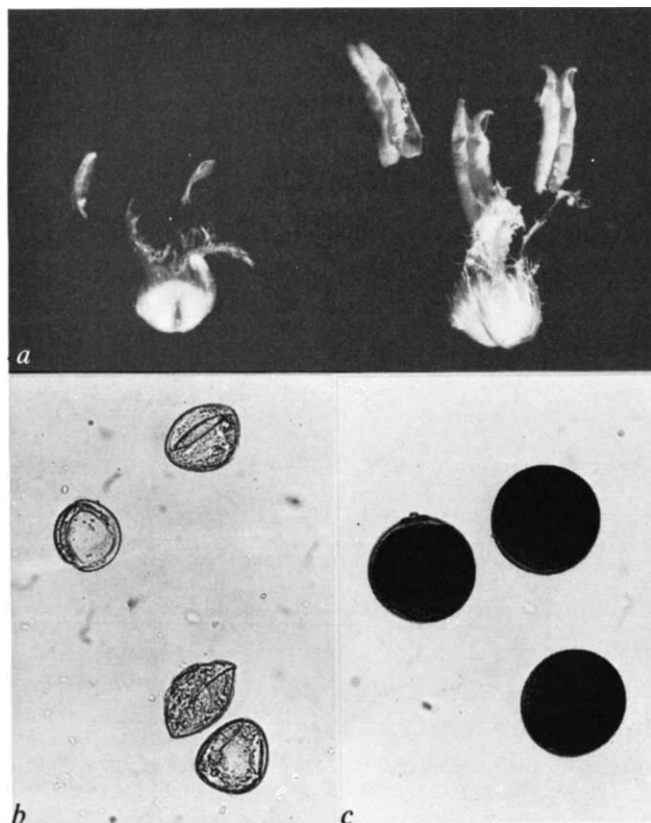


Fig. 1 *a*, Florets excised from copper-deficient (left) and copper-sufficient wheat plants. The copper-deficient anthers are much smaller than normal and the ovule is slightly smaller; one anther is still attached to the receptacle. The filaments do not elongate. ($\times 5$). *b* and *c*, Pollen grains (treated with iodine) from copper-deficient (*b*) and copper-sufficient (*c*) wheat plants. The copper-sufficient grains stain strongly and are 55–60 μm in diameter; copper-deficient grains are 40–45 μm in diameter and do not stain. Wheat plants (*Triticum aestivum* cv. Halberd) were grown in a copper-deficient siliceous sand from Tintinara, South Australia (Laffer sand) in an evaporatively cooled glasshouse. Each pot contained 12 kg of soil which supported three plants sown on July 2, 1974. Appropriate amounts of nutrients N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, Mo and Cl were added with or without Cu (at 4 mg per pot). Plants supplied with copper grew normally while copper-deprived plants showed typical deficiency symptoms: wilting, wither-tip, rat-tailed ears, delayed maturity and failure to set grain (first reported by Lipman and Mackinney¹).

meiosis in relation to the time of application of copper, and if confirmed, would explain why a supply of copper, just sufficient for vegetative growth, may be inadequate for the reproductive phase.

An alternative hypothesis is that non-viability is caused by the lack of accumulation of starch in the developing pollen. This could be related to low soluble carbohydrate levels in copper-deficient plants (R. D. G., unpublished). Because the responsiveness to copper changes quite suddenly, however, it seems that the effect is occurring at a more critical stage of pollen formation than starch accumulation.

These findings may assist cytological and biochemical studies of microsporogenesis as well as being of potential practical use for the production of hybrid seed. It should prove possible in reproducible and well defined low copper environments, both in the field and in the glasshouse, to grow wheat, and probably other species, which are consistently male sterile. This would be especially useful where emasculation is unusually difficult or time-consuming.

Environmental conditions such as moderate water, temperature or nutritional stress can induce male sterility in genetically normal plants. Copper deficiency is a good example, and similar responses to boron deficiency have been reported⁶.

This type of plant response may be important in their evolution as, by the increased possibility of cross pollination caused by male sterility in a harsh environment, adaptation would be more rapid.

R. D. GRAHAM

Waite Agricultural Research Institute,
The University of Adelaide,
Glen Osmond, South Australia 5064

Received January 3, 1975.

- ¹ Lipman, C. B., and Mackinney, G., *Pl. Physiol. Lancaster*, **6**, 593–599 (1931).
- ² Riceman, D. S., and Donald, C. M., Pamphlet 78, Council for Scientific and Industrial Research, Melbourne, Australia (1938).
- ³ Chaudhry, F. M., and Loneragan, J. F., *Aust. J. agric. Res.*, **21**, 865–879 (1970).
- ⁴ Hauser, E. J. P., and Morrison, J. H., *Am. J. Bot.*, **51**, 748–752 (1964).
- ⁵ Bennett, M. D., Chapman, V., and Riley, R., *Proc. R. Soc.*, **B178**, 259–275 (1971).
- ⁶ Lohnis, M. P., *Meded. LandbHoogesch.*, Wageningen., **41**, (1937); **44** (1940).

Elimination of mycoplasmas from cell cultures with sodium polyanethol sulphonate

MYCOPLASMAS are common contaminants of cell cultures, though rarely of primary cultures^{1–3}. The organisms can have a variety of effects on cells in cultures³. They can severely interfere with the metabolism of the cells. Depletion of the culture medium content of arginine causing chromosomal changes and morphological alteration of the cells have been observed, although cytopathogenic changes similar to those caused by viruses are less common. The use of cell cultures contaminated with mycoplasmas may suppress the yield of viruses, but occasionally enhance it. Since some species of *Mycoplasma* may haemadsorb and haemagglutinate, the presence of such organisms in cell cultures may be mistaken for that of certain viruses.

Methods to eradicate mycoplasmas from cell cultures³ are usually of restricted value, since the organisms, although not detectable for some time, usually reappear. Such methods include treatment of the cultures with antibiotics⁴, and/or antisera homologous to the contaminating species of *Mycoplasma*³. Heating of the cultures at 41 °C or treatment with N-tris-(hydroxymethyl)methylglycine (TRICINE) buffer has also been reported⁷. An intracellular localisation of the mycoplasmas would explain why contaminated cell cultures are refractory to treatment, but it is not yet known whether mycoplasmas do occur intracellularly.

Sodium polyanethol sulphonate (SPS) is an anticoagulant, which also inhibits the bactericidal activity of serum^{8,9}, and is therefore used in transport media for blood specimens to be cultured for bacteria. A 5% solution of SPS impregnated on paper disks inhibits the growth on solid medium of the vast majority of species of *Mycoplasma*, but not those of *Acholeplasma*⁴. SPS (Koch-Light) was used for eliminating mycoplasmas contaminating cell cultures of the following contaminated cell lines. Human embryonic lung fibroblasts (HEL), HeLa (strain S3), MASA (originally derived from bone marrow¹⁰) and McCoy cells.

SPS (10%) was sufficient to eradicate the mycoplasmas from the HeLa and McCoy cell lines after incubation for 22 h (Table 1). The cultures remained free of mycoplasmas within the 4 months of follow-up. The HEL cells were more susceptible to SPS and did not survive treatment for 6 h with a 5% solution. Lower concentrations of SPS were not enough to kill the mycoplasmas in the HEL cell cultures. As for the MASA cells, the mycoplasmas could not be eradicated by one treatment with SPS in concentrations which did not also kill the cells. The mycoplasmas were, however, reduced by 10⁴ colony-forming units (CFU ml⁻¹) in the culture medium by the first treatment. After treated a second time with 10% SPS for 6 h the cell cultures were free from mycoplasmas and remained so for at least 4 months.

Eradication of the mycoplasmas was successful only when the culture bottle was completely filled with the mixture of the

Table 1 Susceptibility of some cell lines and of contaminating mycoplasmas to SPS

Cells	SPS (%)	Period of treatment (h)	Mycoplasmas eliminated	Cell line killed
HeLa	5	22	—	—
	10	22	+	—
McCoy	5	22	—	—
	10	22	+	—
HEL	2	6	—	—
	5	6	NT†	+
MASA	5	6	—	—
	10	6	—*	—
	10	22	NT†	+

*The treatment reduced the number of mycoplasmas in the culture medium with 10^4 CFU ml⁻¹. Repeated treatment with 10% SPS for 6 h after the cells had been passaged, eliminated the mycoplasmas completely from the cell culture for at least 4 months.

†Not tested.

Cells were grown in MEM (Flow) with foetal calf serum (final concentration 10%), and propagated with the aid of versene. Before and after treatment with SPS, cultures were tested for mycoplasmas in serial dilutions of the tissue culture medium in liquid mycoplasma medium, and by direct inoculation on to agar plates¹¹. The culture medium specimens tested also contained cells. In some instances, the cell suspensions were also frozen and thawed before culture for mycoplasmas. Mycoplasmas isolated from the cultures before treatment belonged to the *M. orale* (type 1) species. The culture medium contained at least 10^6 colony-forming units (CFU) of *Mycoplasma* ml⁻¹ before treatment with various concentrations of SPS, at which time they produced a confluent layer of cells in Carrel bottles. The mixtures were incubated at 37 °C for various periods after which the cells were passed to new bottles. Attempts were made to isolate mycoplasmas before each new passage of the cells. If not stated otherwise, the results presented are based on those obtained from a single treatment with SPS.

culture medium and the SPS. The treatment also killed a large proportion of the cells, but did not disturb further propagation of the cell lines. Cells cultured in suspension proved more susceptible to SPS than when the corresponding cells were cultured in monolayer.

In many laboratories the majority of cell lines used are presumably contaminated with mycoplasmas. We found mycoplasmas in more than 60% of a series of 400 specimens of cell culture media from such cultures. The route of infection of cell cultures with mycoplasmas is often obscure, although contamination of the cultures by mycoplasmas from the respiratory tract of the persons handling the cultures and the use of contaminated constituents of the cell culture medium, particularly serum, have been considered the commonest sources of infection. The former may be suspected for the cell cultures we studied, since the mycoplasmas isolated belonged to the species of *M. orale* (type 1).

The mechanism by which SPS kills mycoplasmas is not known, but it is probably by lysis. The presence of cholesterol in the cytoplasmic membrane of mycoplasmas but not that of achleplasmas would explain the difference in susceptibility to this compound, the former organisms being more susceptible. When tested with the disk-diffusion technique a 5% solution of SPS did inhibit mycoplasmas, with the exception of certain strains of *M. anatis*, *M. gallinarum*, *M. gatae*, *M. iners* and *M. maculosum*, but not achleplasmas⁴. The mycoplasmas isolated from the cell cultures we studied belonged to one species, *M. orale* (type 1). The susceptibility to SPS of the great number of other species of *Mycoplasma* known to contaminate cell cultures is to be tested. The susceptibility of *M. orale* (type 1) does not seem to differ, however, from that of the majority of other species of *Mycoplasma*⁴. The presence of cholesterol in the cytoplasmic membrane of mycoplasmas and its absence in that of achleplasmas has also been offered as an explanation of the differential effect of lyssolecithin on these organisms¹². We also tested whether lyssolecithin could

be used for eliminating mycoplasmas from cell cultures, but the differential between the concentrations that killed the mycoplasmas and the cells proved too small for lyssolecithin to be of use for this purpose, at least for the cell lines studied. This also seems to hold for certain cell lines with SPS.

PER-ANDERS MÅRDH

*Institute of Medical Microbiology,
University of Lund,
Sölvegatan 23,
S-223 62 Lund, Sweden*

Received December 6, 1974; revised February 24, 1975.

- Barile, M. F., *INSERM*, 33, 135 (1974).
- Barile, M. F., Hopps, H. E., Grabowski, M. W., Riggs, D. B., and Del Giudice, R. A., *N.Y. Acad. Sci.*, 225, 251 (1973).
- Kenny, G. E., *Contamination in Tissue Culture*, 107 (Academic, New York, 1973).
- Freundt, E. A., Andrews, B. E., Ernø, H., Kunze, M., and Black, F. T., *Zbl. Bakt. Hyg. I. Abt. Org.*, A225, 104 (1973).
- Hayflick, L., *Nature*, 185, 783 (1960).
- Burkshirk, H. H., *Appl. Microbiol.*, 15, 1442 (1967).
- Spendlove, R. S., *Proc. Soc. exp. Biol. Med.*, 137, 258 (1971).
- Lowrance, B. L., and Traub, W. H., *Appl. Microbiol.*, 17, 839 (1969).
- Rosner, R., *Appl. Microbiol.*, 19, 281 (1970).
- Kjellén, L., *Virology*, 18, 64 (1962).
- Mårdh, P.-A., and Weström, L., *Br. J. vener. Dis.*, 46, 179 (1970).
- Mårdh, P.-A., and Taylor-Robinson, D., *Med. Microbiol. Immun.*, 158, 219 (1973).

Penicillin-binding proteins and cell shape in *E. coli*

β-LACTAM antibiotics (penicillins and cephalosporins) have attracted considerable attention as probes of cell growth and division^{1,2}. Although extensive studies have been made on both penicillin-sensitive enzymes and penicillin binding proteins³, there has been no clear indication of the role of any of these components in the effects of β-lactam antibiotics on cell growth. We report the identification of a minor penicillin binding protein which we believe to be the target at which the amidinopenicillanic acid designated FL1060 (ref. 4) acts to affect the shape of *Escherichia coli*. This is the first example of the identification of a penicillin binding protein with an essential and defined role in bacterial cell growth.

Whereas low concentrations of typical β-lactam antibiotics specifically inhibit cell division in *E. coli*, FL1060 at its lowest effective concentration causes the conversion of *E. coli* rods into ovoid shaped cells^{4,5}. The atypical effects of FL1060 are paralleled by its failure markedly to inhibit any of the three known penicillin-sensitive enzymes of *E. coli*^{5,6} and by its apparent failure to compete with the binding of ¹⁴C-benzylpenicillin to *E. coli* membranes⁶. As these experiments measured total enzyme, or total binding, it is still possible that FL1060 inhibits a minor enzyme species or competes with a minor penicillin-binding protein in the cell envelope. We have re-examined the binding of FL1060 to the *E. coli* penicillin binding proteins using an improved method for the detection of the individual binding proteins, and have shown that FL1060 does indeed compete with high affinity for a minor penicillin binding protein in the *E. coli* inner membrane.

¹⁴C-benzylpenicillin (54 mCi mmol⁻¹; Searle Amersham) was bound to purified membranes of *E. coli* K12 (strain KN126 obtained from Dr T. Nagata). The membrane proteins were solubilised and fractionated on sodium dodecyl sulphate (SDS) polyacrylamide slab gels. As conventional autoradiography was too insensitive to allow rapid detection of the binding proteins, they were detected by incorporating the scintillant 2,5-diphenyloxazole (PPO) into the gel matrix (fluorography⁷) and exposing the dried gel to Kodak RPRoyal X-ray film at -70 °C (ref. 7). Figure 1a shows the pattern of six penicillin-binding proteins consistently obtained from the membranes of *E. coli* K12. As all six proteins are found exclusively in the inner (cytoplasmic) membrane (our unpublished results) we routinely removed the outer membrane after binding ¹⁴C-benzylpenicillin (taking advantage of the insolubility of the outer membrane in 1% Sarkosyl at room temperature⁸) and fractionated only the

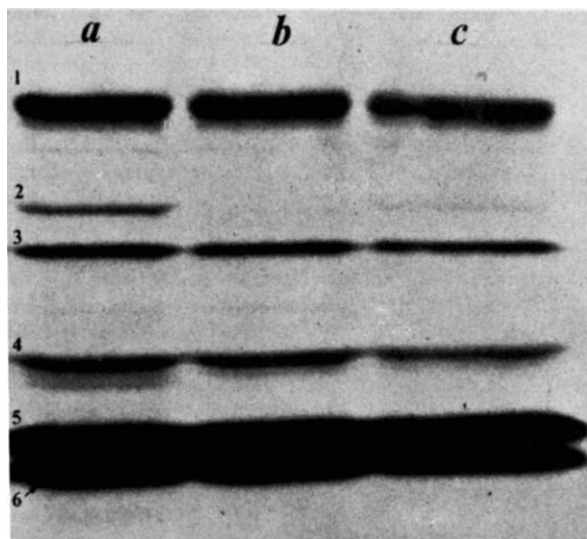


Fig. 1 Competition of FL1060 and 6-APA for penicillin-binding proteins. Membranes were prepared from *E. coli* KN126 growing exponentially in Difco antibiotic medium No. 3 as described previously⁹, washed twice in 0.01 M sodium phosphate buffer, pH 7.0, and resuspended at 20 mg ml⁻¹ in 0.05 M sodium phosphate buffer, pH 7.0; 10 mM MgCl₂. To assay penicillin binding proteins 20 µl of ¹⁴C-benzylpenicillin (50 µCi ml⁻¹, specific activity, 54 mCi mmol⁻¹) was added to 200 µl of washed membranes for 10 min at 30 °C. The final concentration of benzylpenicillin was 34 µg ml⁻¹, sufficient to saturate the binding proteins in 10 min at 30 °C. The reaction was stopped and the inner membrane was solubilised by addition of 10 µl of 20% (w/v) Sarkosyl (Geigy) and 5 µl of cold benzylpenicillin (120,000 µg ml⁻¹). After 20 min at room temperature the samples were centrifuged at 100,000g for 40 min and 100 µl of the supernatant was added to 50 µl of SDS buffer (0.2 M Tris-HCl, pH 6.8; 3% SDS; 30% glycerol; 0.002% bromophenol blue). After addition of 15 µl of β-mercaptoethanol, the samples were heated at 100 °C for 2 min and 50 µl was loaded on a 12% discontinuous SDS polyacrylamide slab gel using the buffer system and apparatus described by Laemmli and Favre¹⁰. After fixing the gel, the scintillant PPO was incorporated into the gel and the binding proteins were detected by fluorography⁷ on Kodak RPRoyal X-ray film for 23 d at -70 °C. *a*, Membranes preincubated with 10 µl of distilled water for 10 min at 30 °C. *b*, Membranes preincubated with 10 µl of FL1060 (100 µg ml⁻¹) for 10 min at 30 °C (final concentration of FL1060 was 5 µg ml⁻¹). *c*, Membranes preincubated with 10 µl of 6-APA (1 mg ml⁻¹) for 10 min at 30 °C (final concentration of 6-APA was 50 µg ml⁻¹). Electrophoresis was from the top to the bottom of the figure.

Sarkosyl-soluble inner membrane. As radioactive FL1060 is not readily available, we have studied the binding of FL1060 to the penicillin binding proteins by competition with ¹⁴C-benzylpenicillin. Figure 1*b* shows that prebinding FL1060 (5 µg ml⁻¹) for 10 min at 30 °C resulted in the complete blocking of the subsequent binding of ¹⁴C-benzylpenicillin to binding protein 2 without affecting any other binding protein. A more detailed analysis showed that FL1060 (0.013 µg ml⁻¹) produced a 50% inhibition of the binding of a saturating concentration of ¹⁴C-benzylpenicillin to binding protein 2. This is in good agreement with the minimal inhibitory concentration (MIC) of 0.05 µg ml⁻¹ required to inhibit the growth of this strain. A similar blocking of binding to protein 2 was observed if ¹⁴C-benzylpenicillin was added to membranes prepared from cells grown for 60 min in the presence of FL1060 at 0.1 µg ml⁻¹.

To implicate binding protein 2 further in the effects of FL1060 on cell shape we screened a number of penicillins and cephalosporins to find those which produced similar morphological effects to those produced by FL1060. Among the compounds screened, only the penicillin precursor molecule 6-aminopenicillanic acid (6-APA) resulted in the production of ovoid cells at the lowest effective concentrations, and at no concentration did it produce the specific inhibition of cell division typical of most β-lactam antibiotics. 6-APA was

markedly inferior to FL1060 in its action on cell shape as it was only effective at relatively high concentrations (MIC 14 µg ml⁻¹) and over a narrow concentration range above which lysis occurred. Figure 1*c* shows that as expected 6-APA at 50 µg ml⁻¹ completely inhibited the binding of ¹⁴C-benzylpenicillin to binding protein 2. A concentration of 2 µg ml⁻¹ 6-APA produced 50% inhibition of binding to protein 2. Significant inhibition of binding proteins 1, 3 and 4 was also produced by 6-APA at 50 µg ml⁻¹. This is probably the cause of cell lysis by 6-APA as the binding of β-lactams to these latter proteins is thought to be the cause of the effects of typical β-lactam antibiotics on cell growth and division (our unpublished results).

Binding protein 2 has an apparent molecular weight of 66,000 as measured by its relative mobility on SDS polyacrylamide slab gels compared with that of 7 standard proteins, and constitutes 0.5% of the total penicillin binding at saturating concentrations of ¹⁴C-benzylpenicillin. This represents about 10 copies of binding protein 2 per cell. Typical β-lactam antibiotics that affect cell division and cause cell lysis either fail to compete with binding protein 2 (for example, most cephalosporins) or bind with low affinity. These latter antibiotics do not result in the production of ovoid cells as they bind to other proteins thought to be involved in cell division and cell elongation with greater affinity than they do to binding protein 2 (our unpublished results).

We believe that penicillin-binding protein 2 is the target at which FL1060 acts to affect the shape of *E. coli*. The protein is presumably an enzyme involved in the terminal stages of peptidoglycan metabolism. At present it is not clear whether this is a previously unknown enzyme or a minor component of one of the established penicillin-sensitive enzymes³. As FL1060, unlike typical β-lactam antibiotics, is much more effective against Gram-negative than Gram-positive organisms it has been suggested that it acts on a target which is unique to the former class of organisms³ (R. James, J. Haga and A.B.P., unpublished).

We thank Dr F. Lund of Leo Laboratories for FL1060. This work was supported by a grant from the National Cancer Institute.

BRIAN G. SPRATT
ARTHUR B. PARDEE

Moffett Laboratories,
Department of Biochemical Sciences,
Princeton University,
Princeton, New Jersey 08540

Received January 24, 1975.

- Schwarz, U., Asmus, A., and Frank, H., *J. molec. Biol.*, **41**, 419-429 (1969).
- Donachie, W. D., and Begg, K. J., *Nature*, **227**, 1220-1224 (1970).
- Blumberg, P. M., and Strominger, J. L., *Bact. Rev.*, **38**, 291-335 (1974).
- Lund, F., and Tybring, L., *Nature new Biol.*, **236**, 135-137 (1972).
- Park, J. T., and Burman, L., *Biochem. biophys. Res. Commun.*, **51**, 863-868 (1973).
- Matsushashi, S., et al., *J. Bact.*, **117**, 578-587 (1974).
- Bonner, W. M., and Laskey, R. A., *Eur. J. Biochem.*, **46**, 83-88 (1974).
- Filip, C., Fletcher, G., Wulff, J. L., and Earhart, C. F., *J. Bact.*, **115**, 717-722 (1973).
- Inouye, M., and Guthrie, J. P., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 957-961 (1969).
- Laemmli, U. K., and Favre, M., *J. molec. Biol.*, **80**, 575-599 (1973).

Immunological and bacteriological basis for vaccination against dental caries in rhesus monkeys

A REPRODUCIBLE model for dental caries has been established in rhesus monkeys¹. The development of caries is comparable to that found in man because caries is induced by maintaining the animals on a human type of diet, this is associated with an overgrowth of a naturally acquired *Streptococcus mutans* and caries develops in teeth that are morphologically similar to human teeth. The incidence of

smooth surface caries was reduced by subcutaneous or submucous immunisation with a heat-killed *Strep. mutans* in Freund's incomplete adjuvant (FIA)². Protection has been correlated predominantly with the rate of development of serum complement fixing antibodies to a hydroxyl apatite fraction of the culture supernatant (HACS) of *Strep. mutans*. The aims of this investigation were to attempt to elucidate the mechanism of prevention of dental caries in rhesus monkeys by immunisation with a monkey passaged *Strep. mutans*. The relative effects of saliva and crevicular fluid on *Strep. mutans* were tested by developing a differential sampling technique across the plaque and the adjacent saliva and crevicular fluid. This revealed that the very low caries score in the immunised animals was correlated with serum antibodies and with a reduction in the number of *Strep. mutans* in crevicular fluid and the adjacent bacterial plaque zone.

A series of 11 young rhesus monkeys were caged, examined and maintained on a human type of diet, containing 15% sucrose as described previously¹. All animals had a fully erupted deciduous dentition but no permanent teeth and their ages were assessed to range from 11 to 21 months (ref. 3). The animals were examined at monthly intervals and a steady increase in weight was recorded in all animals.

A streptomycin resistant *Strep. mutans* (serotype c) was successfully implanted into the mouth of another rhesus monkey, then reisolated and a heat-killed vaccine containing about 10^9 of the passaged *Strep. mutans* per ml was prepared and used as described previously². The animals were randomly distributed into three groups and there was a comparable sex distribution in each of these groups. One

group of three monkeys was given the first subcutaneous injection of 0.5 ml of the vaccine in an equal volume of FIA into the contralateral limbs. This was followed by four injections of 1 ml of the vaccine alone at weeks 4, 8, 16, and 56. The adjuvant control group of four animals was given subcutaneous injections of 1 ml of FIA, followed by four saline injections; the saline control group of four animals was given five subcutaneous injections of saline at the same time intervals.

Blood was taken from the femoral vein at monthly intervals and always before immunisation, and the serum was stored at -20°C . The blood indices remained within the normal range throughout the investigation. Serum antibodies were assayed by the complement fixation (CF) test in microplates, as described previously⁴. The immunised group showed a rapid increase in CF antibodies from a mean baseline titre of $\log_2$ 1-4 within four weeks and reached a maximum of $\log_2$ 6 by week 24 (Fig. 1). A small decrease in titre followed, but the booster injection at week 56 induced a secondary antibody increase to $\log_2$ 6. Both control groups had a very low serum antibody titre of less than $\log_2$ 3.

Whole saliva was collected at monthly intervals, after subcutaneous injection of 0.5 mg per kg of pilocarpine¹, and the haemagglutinating antibodies were determined by a modified micromethod⁴. The immunised group showed a modest increase from a mean titre of $\log_2$ 1.2-2.3 and remained between $\log_2$ 2-3 from week 4 to 84. Both control groups showed little initial change but from week 24 to 48 titres between $\log_2$ 1 and 2 were found in the saline group and between $\log_2$ 2 and 3 in the adjuvant group.

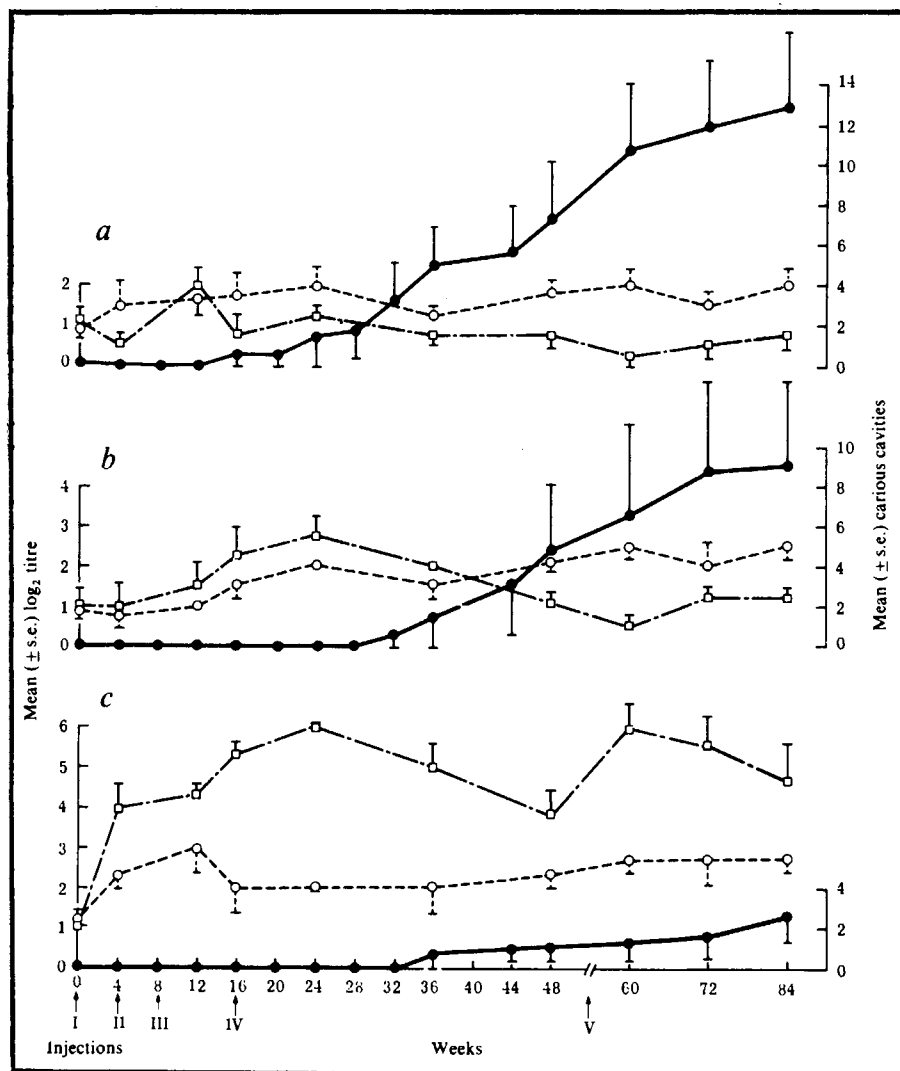


Fig. 1 Sequential serum and salivary antibody responses to immunisation and caries score over a period of 84 weeks. a, Saline group; b, adjuvant group; c, *Strep. mutans*/adjuvant group. ●, Caries index; □, serum antibodies; ○, salivary antibodies.

Clinical and X-ray examination for dental caries was performed at monthly intervals and caries was scored as described previously¹. The mean onset of smooth surface caries was 28.5 (± 4.6) weeks in the saline group, 54.5 (± 10.8) weeks in the adjuvant group and more than 55 weeks in the immunised group (one animal had no caries by week 84). Both control groups showed a rapid increase in smooth surface caries (Fig. 1) and by week 84 a mean of 12.75 (± 3.6) cavities per animal was found in the saline group and 9.0 (± 4.3) cavities in the adjuvant group of monkeys. In contrast the immunised group had developed only 2.7 (± 1.4) cavities per animal. The results suggest that immunisation had delayed the onset of caries and had decreased both the rate of development and the incidence of caries. The serum CF antibodies seemed to be associated with this protection, as was found previously². Fissure caries appeared from week 20 in the saline and week 28 in the adjuvant group; by week 84 there were 2.0 (± 1.3) and 2.5 (± 1.0) cavities per animal respectively. Fissure caries was not found in the immunised group by week 84.

No attempt was made to implant *Strep. mutans* into any of the animals. A sequential analysis of randomly pooled plaque, from the buccal and approximal surfaces of teeth was carried out at monthly intervals from 0 to 6 months. Samples of plaque were placed into transport medium and then cultured, under anaerobic conditions, on TYC medium⁵. *Strep. mutans* and *Strep. sanguis* were identified by their colonial morphology, production of dextran and carbohydrate fermentation reactions. *Strep. mutans* (serotype c) was isolated from dental plaque from week 4 and all animals harboured a large number of these *Streptococci* from week 24 to 84. The mean time of isolation of *Strep. mutans* from the saline group was 15.5 (± 5.0) weeks, from the adjuvant group 19.5 (± 1.5) weeks and from the immunised group 20 (± 2.0) weeks. In contrast, *Strep. sanguis* was isolated from all animals between 0 and 4 weeks and persisted throughout the experimental period.

Once the *Streptococci* were established in all animals, a quantitative analysis was performed, to find out if immunisation had any effect on colonisation by *Strep. mutans*. Weighed samples of plaque were collected in 2 ml of transport medium, at monthly intervals, from 6 to 12 months. The samples were dispersed by shaking vigorously with glass beads and then plated out in serial tenfold dilutions on TYC medium. The results were expressed as a percentage of colony forming units (CFU) of *Strep. mutans* and *Strep. sanguis*, in terms of the total number of *Streptococci* per mg of plaque grown on the TYC medium. Little difference was found between the mean CFU of *Strep. mutans* of saline injected (67 ± 7.7), adjuvant treated (46 ± 9.4) and immunised (54 ± 15.0) animals. In view of this and the fact that serum and not salivary antibodies seem to be associated with protection against caries both in rhesus monkeys² and in man⁴, the question was posed whether protection was mediated by serum through crevicular fluid. The hypothesis to be tested was that according to the immune state of the animal there should be a quantitative difference in *Strep. mutans* in the 4 zones adjacent to the tooth (Fig. 2); crevicular fluid, crevicular fluid plaque and salivary plaque zones and saliva.

An aliquot of 0.1 ml of the whole saliva was placed directly into transport medium. The salivary plaque zone was collected by lightly removing with a probe all plaque from about the cervical third of the buccal surfaces of the left maxillary molars and placing this into transport medium. The 'crevicular fluid-plaque zone' was sampled by rubbing the blunt end of a sterile root canal paper point against the surface from which the 'salivary-plaque zone' had just been removed and cutting off the end of the paper point into transport medium. Finally, crevicular fluid was collected from the same site by placing the needle of a 50 μ l syringe between the two deciduous molars and flush-

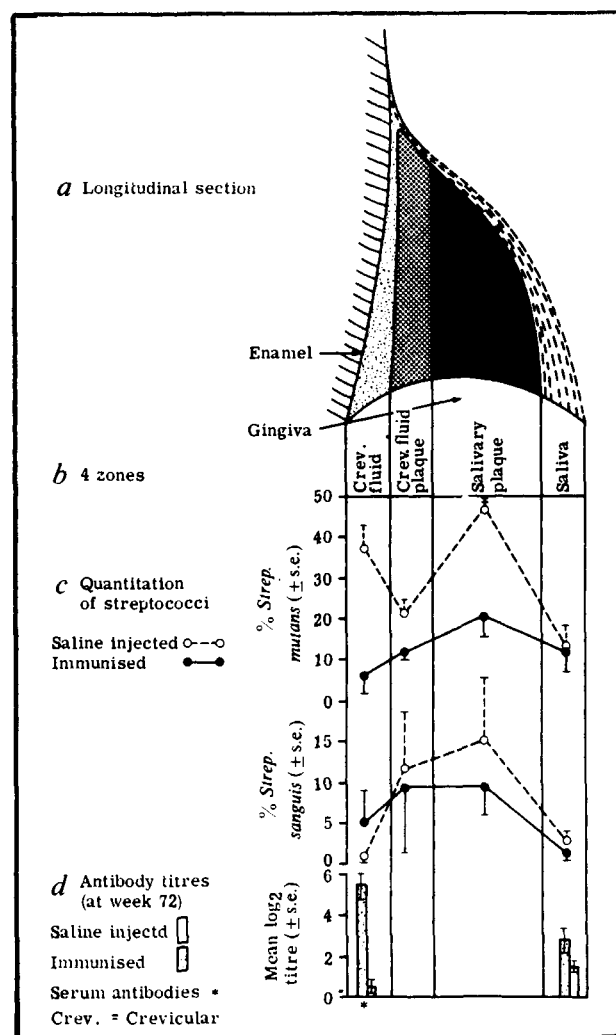


Fig. 2 Salivary and crevicular fluid immune mechanisms affecting *Strep. mutans* in the four zones adjacent to the tooth.

ing through and immediately aspirating 10 μ l of transport medium⁶; this was repeated and the washings were collected into transport medium. The four specimens were suitably diluted and the CFU of *Strep. mutans* and *Strep. sanguis* were determined monthly between 12 and 18 months. The results were expressed by comparing the sum of the means ($\pm$ s. e.) of the six serial determinations in each animal in one group with those in another group (Fig. 2). Although three of the four sampling sites showed a lower percentage of *Strep. mutans* in the immunised than control groups this reached significant levels only in crevicular fluid ($t=4.035$; $P<0.02$) and the crevicular fluid-plaque zone ($t=2.77$; $P<0.05$). The results for the adjuvant group were intermediate between the saline and immunised groups. A significant difference in the corresponding results with *Strep. sanguis* was not found; indeed there were about nine times more *Strep. sanguis* in the crevicular fluid of immunised than saline injected animals. It is also of considerable interest that the crevicular fluid of saline injected animals yielded about 60 times more CFU of *Strep. mutans* (38) than *Strep. sanguis* (0.6), whereas the corresponding ratio in the immunised animals was about 1:1, or 6 CFU in each group. Preliminary determinations of the percentage of *Strep. mutans* in terms of the total anaerobic bacterial growth on blood agar showed that crevicular fluid of the saline injected group yielded about 25 times more *Strep. mutans* than the immunised group.

There are two mechanisms which may be involved in

prevention of caries: first salivary IgA preventing adherence of *Strep. mutans*⁷, and second, crevicular fluid mediated serum antibodies and blood leucocytes inhibiting *Strep. mutans* or its products, suggested here. The relative roles of saliva versus crevicular fluid were put to the test by developing a differential sampling technique across the plaque and the adjacent two fluid layers. This revealed that the very low caries score in the immunised animals was correlated with a low percentage of *Strep. mutans* in crevicular fluid and the crevicular fluid-plaque zone and an increased serum antibody titre to *Strep. mutans* (Fig. 2). A similar relationship was not found with salivary haemagglutinating antibodies and *Strep. mutans* in saliva and the salivary-plaque zone which probably constitutes the bulk of dental plaque as tested conventionally. A significant reduction in *Strep. mutans* in plaque of immunised animals was not found by others⁸⁻¹⁰, with the exception of one out of seven groups of animals in one series.¹⁰ It is assumed that the immune responses in crevicular fluid are mediated by serum antibodies and possibly leukocytes, but this will have to be tested directly on crevicular fluid. The bacteriological results are consistent with the hypothesis that the immune components of crevicular fluid are responsible for protection against smooth surface caries, and that crevicular fluid may act as a functional analogue of blood.

T. LEHNER
S. J. CHALLACOMBE
JILL CALDWELL

Department of Oral Immunology and Microbiology,
Guy's Hospital Medical and Dental Schools,
London SE1 9RT, UK

Received January 30; revised February 18, 1975.

- ¹ Lehner, T., Challacombe, S. J., and Caldwell, J., *Archs oral Biol.* (in the press).
- ² Lehner, T., Challacombe, S. J., and Caldwell, J., *Archs oral Biol.* (in the press).
- ³ Hurme, V. O., *Ann. N.Y. Acad. Sci.*, **85**, 795 (1960).
- ⁴ Challacombe, S. J., Guggenheim, B., and Lehner, T., *Archs oral Biol.*, **18**, 657 (1973).
- ⁵ de Stoppelaar, J. D., van Houte, J., and Backer Dirks, O., *Caries Res.*, **3**, 190 (1969).
- ⁶ Skapski, H., and Lehner, T., *Int. Assoc. dent. Res.* (Abstract, in the press).
- ⁷ Genco, R. J., Evans, A. T., and Taubman, M. A., *Adv. exp. Med. Biol.*, **45**, 327 (1974).
- ⁸ Bowen, W. H., *Br. dent. J.*, **126**, 159 (1969).
- ⁹ Tanzer, J. M., Hageage, C. J., Jr., and Larson, R. H., *Archs oral Biol.*, **18**, 1425 (1973).
- ¹⁰ Taubman, M. A., and Smith, D. J., *Infect. Immun.*, **9**, 1079 (1974).

Photopigment conversions expressed in receptor potential and membrane resistance of blowfly visual sense cells

IN spite of the fact that intracellular recordings from photoreceptor cells have been possible for many years and knowledge regarding the photochemistry of these cells has been steadily increasing, it is still unclear how resistance changes and potential changes of the cell membrane are mediated by photoconversion of the pigments located in that membrane. It is generally supposed that a link does exist between the photochemistry and the membrane phenomena, but evidence is sparse.

Recent evidence is provided by the work of Hochstein *et al.*¹ on the barnacle and Minke *et al.*² on *Limulus* who demonstrated that light-evoked changes in resistance and potential of the receptor cell membrane depend on the photopigment conditioning. Their experiments are founded on two crucial facts in the photochemistry of these and many other invertebrate species. First, the photopigment has two thermostable states instead of one, and second, practically all pigment can be converted into either the rhodopsin or the metarhodopsin state when illuminated with carefully chosen wavelengths. It proved that stimulation with a metarhodopsin-generating wavelength of a receptor cell harbouring mainly rhodopsin, caused resistance and potential changes which persisted in the dark and could only be undone by a wavelength causing renewed rhodopsin production.

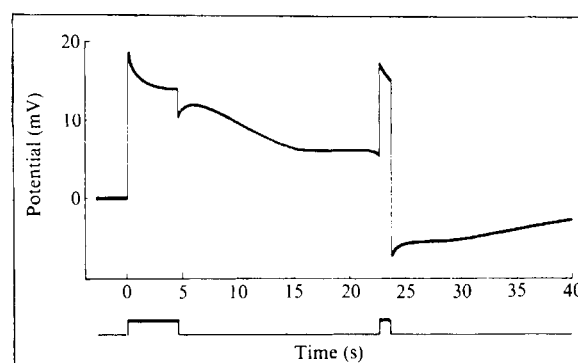


Fig. 1 Upper trace, membrane potential of a visual receptor cell (blowfly wild type) measured intracellularly with a glass microelectrode. Lower trace, light stimulus. Positive deflection represents membrane depolarisation. The cell has been pre-adapted to intense red light (603 nm). The first stimulus shown in the figure, delivered after 2 min in the dark, is a blue flash (460 nm), causing a depolarisation. Apart from a short dip, switching off the blue light does not change the light adapting course of the receptor potential. A flash of red light (second stimulus in the figure) depolarises the membrane again and induces normal repolarisation at its termination.

For the blowfly, the particulars of rhodopsin and metarhodopsin are given by Stavenga *et al.*^{3,4} who described optical work on the pupil mechanism of this species. Their results point in the same direction as those of barnacle and *Limulus*, if their hypothesis of the pupil pigment granules migrating in an intracellular force field created by the resistance changes, (which are simultaneously giving rise to the receptor potential) is correct.

Hence, we have attempted to demonstrate in the blowfly a link between the photoconversions and the light-induced changes in resistance and potential of the photoreceptor membrane. The latter were measured intracellularly in the photoreceptor cells of practically intact specimens, both wild type and the mutant Chalky.

We used the following procedures (Fig. 1). First, stimulation with red light (603 nm) brought nearly all photopigment molecules into the rhodopsin state. After 2 min in the dark, a metarhodopsin-generating blue flash was then presented, inducing a normal receptor potential, that is, a depolarisation of the cell membrane. This was, however, not followed at its termination by a normal quick return to the preillumination level, that is, a repolarisation of the cell membrane. The very slowly repolarising cell membrane could then become depolarised again by a flash of intense red light which, on being turned off, promptly caused membrane repolarisation to the former dark level.

Such a recording of a blowfly wild type, is shown in Fig. 1,

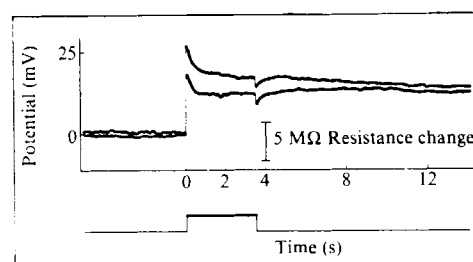


Fig. 2 Upper trace, membrane potential and resistance changes of a visual receptor cell (blowfly wild type). The resistance changes are measured by a square wave (30 Hz) driven bridge circuit. With a balanced bridge a single trace is seen, but it seems to split in two as soon as the bridge becomes unbalanced; the distance between the two "parallel" traces indicates the changed membrane resistance with respect to the preflash value, while the membrane potential is represented by their mean. Lower trace, light stimulus (blue light). The cell has been pre-adapted to red light (603 nm) and blue light is presented after 2 min in the dark. After the blue light is switched off neither the membrane potential nor the resistance returns to the preflash value. The dip in potential is not accompanied by a dip in resistance.

in which termination of the blue flash is marked by a dip in the receptor potential, which otherwise does not seem to be deflected from the slowly repolarising course which normally occurs during dark adaptation. We call this sustained depolarisation a 'tail', following Hochstein *et al.*¹. At the end of the subsequent red flash a rapid repolarisation was induced which markedly exceeded the former dark level (Fig. 1).

We will now compare these data with those obtained for photo-receptor cells of the barnacle² and *Limulus*³. With an essentially similar stimulus programme, but different wavelengths (because of the different absorption spectra of rhodopsin and metarhodopsin in these species), Hochstein *et al.*¹ elicited from the barnacle visual sense cells, tails which differ only slightly from ours. The barnacle tails markedly deflect from the slowly repolarising course normal during light adaptation, by repolarising more rapidly. The blowfly tails are in fact also characterised by a change in repolarising rate, but in the other direction. In *Limulus*, Minke *et al.*³ did not observe any change in repolarising rate.

At the beginning of the tail in the barnacle there is no dip as in blowflies, but a small repolarising step, while *Limulus* has an even smaller step. But apart from these small differences of tail-slope and dip-shape which may well be explained by the differences in photochemistry, it seems certain that the underlying mechanism must be very similar if not the same in the three species.

Following the proposal of Hochstein *et al.*¹ and Minke *et al.*², we suggest the following scheme for the blowfly. A great number of hypothetical particles called "exciters", capable of lowering the membrane resistance, are made by the blue flash as a byproduct of metarhodopsin. These exciters continue exerting their influence on the membrane resistance, thus creating a tail, without being hampered by hypothetical anti-particles called "inhibitors" which are normally present to restore the membrane resistance as soon as the light is switched off. As a result of our experimental procedure these "inhibitors" are lacking because they have all been made ineffective by the preceding 2 min of darkness.

This happens after every such dark period, regardless of the preceding illumination history. But without the special measures taken in our experiment, illumination after the 2-min dark period encounters a mixture of both rhodopsin and metarhodopsin and immediately starts the production of, respectively, metarhodopsin + excitor and rhodopsin + inhibitor, so that the membrane can repolarise as soon as the light is turned off. But as a result of our preceding intense red illumination, all metarhodopsin has been turned into rhodopsin + inhibitor, so after the 2-min dark period there is no metarhodopsin left for the manufacture of new inhibitors and the old inhibitors have all become inactive. Only after extended blue illumination, resulting in a new photopigment equilibrium, is there sufficient metarhodopsin to ensure continuous reversal to rhodopsin + inhibitor, resulting in normal restoration of the membrane resistance and repolarisation of the cell when the light is turned off.

The results of simultaneous recordings of the receptor potential and the membrane resistance changes are shown in Figs 2 and 3. The membrane resistance change should resemble the receptor potential unless an electrogenic pump is present. Figure 2 presents a detail of a similar experiment to that in Fig. 1. The receptor potential is accompanied by measurements of membrane resistance changes which were made with an a.c. square wave driven bridge circuit. After the initial depolarisation and resistance decrease at the onset of illumination, it is seen that during as well as after the blue flash the resistance increases again, even rather more than is necessary to keep pace with the slow repolarisation. The dip in the latter, at the beginning of the tail is not accompanied by a similarly temporary change in resistance.

Neither can this be observed in the recording shown in Fig. 3, which stems from a pupil-less blowfly mutant, the variety *Chalky*. In this particular animal the measured resistance chan-

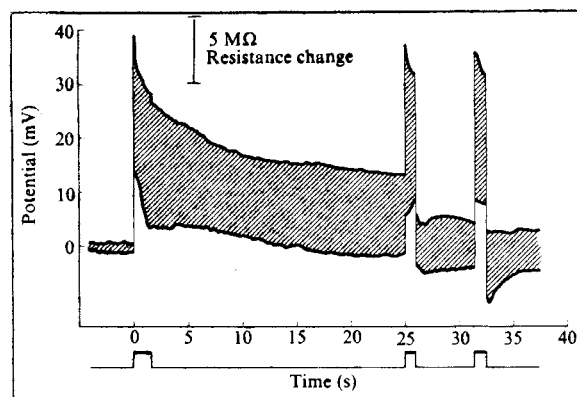


Fig. 3 As in Fig. 2. Measurements on a pupil-less blowfly, mutant *Chalky*. Blue light again causes a depolarisation and a decrease in membrane resistance and when the light is switched off, the cell again retains its light adapting course. Flashes of red light (second and third stimulus) depolarise the membrane as before and are followed by normal repolarisation while the resistance remains short of the former dark value.

ges were much greater than in the one represented in Fig. 2, but still, termination of the blue flash cannot be seen as a change in resistance, though again the dip in the receptor potential is present, even if rather small.

Yet another discrepancy between membrane potential and membrane resistance occurs when the tail is cut short by a red flash: at the cessation of the latter the receptor potential reaches or exceeds the former dark level, while the membrane resistance remains short of its former value.

At just this point a discrepancy also occurs in the barnacle, but here the resistance value exceeds the former dark value even more than the membrane potential does. For *Limulus* there are no data available in the same context. For an explanation of the blowfly and barnacle discrepancies between membrane resistance and membrane potential, the suggestion made by Koike, MackBrown and Hagiwara⁶ of an electrogenic sodium pump which is stimulated by illumination seems a very valuable one.

Although the picture of photochemical conversions leading to electrophysiological events is far from complete as long as the particular nature of the exciters and inhibitors remains unknown, we think that our data support the supposition that a link can be demonstrated between the states of the photopigments (and their conversions into one another) and membrane phenomena. Meanwhile, the lack of a unique relationship between receptor potential and resistance changes might be held to lend support to the concept of an electrogenic sodium pump.

This study was supported financially by the Netherlands Organisation for the Advancement of Pure Research.

Note: added in proof: But even the action of such a pump cannot account for our most recent observation, namely that at the onset of illumination the resistance reaches a new value in less than 5 ms and maintains that value even though the receptor potential falls in 50 ms to half its initial height. On this time scale no pumping mechanism can be held responsible for deviations from a unique relationship, which thus must be discarded.

H. MUIJZER
J. T. LEUTSCHER-HAZELHOFF
D. G. STAVENGA
J. W. KUIPER

Department of Biophysics,
Laboratorium voor Algemene Natuurkunde,
Rijksuniversiteit Groningen,
Groningen,
The Netherlands

Received December 23, 1974; revised February 5, 1975.

¹ Hochstein, S., Minke, B., and Hillman, P., *J. gen. Physiol.*, **62**, 105-128 (1973).
² Minke, B., Hochstein, S., and Hillman, P., *J. gen. Physiol.*, **62**, 787-791 (1973).

- ³ Stavenga, D. G., Zantema, A., and Kuiper, J. W., in *Biochemistry and Physiology of Visual Pigments*, 175–180 (Springer, Berlin, 1973).
⁴ Stavenga, D. G., Flokstra, J., and Kuiper, J. W., *Nature*, (in the press).
⁵ Koike, H., MackBrown, H., and Hagiwara, S., *J. gen. Physiol.*, 57, 723–727 (1971).

Axonal wiring and polarisation sensitivity in eye of the rock lobster

ARTHROPOD compound eyes consist typically of square or hexagonal facets beneath each of which lies a group of photoreceptor cells. Each photoreceptor or retinula cell gives rise to a microvillar fringe, called the rhabdomere, which consists of parallel tubules containing light-absorbing visual pigments. Axons arising from the retinula cells of each group are usually arranged in distinct bundles. These pass through the basement membrane and enter the first optic ganglion, the lamina, where they synapse with second-order neurones.

A modified organisation has been found only in Diptera¹ and now in Decapoda: in both these unrelated orders of arthropods, retinula cell axons interweave before they terminate on axons of the ganglion cells in the lamina cartridges. Whereas in Diptera this rather special arrangement is generally correlated with the 'open' type of rhabdom² (that is, each rhabdomere has its own visual axis and interweaving of retinula axons of several complete visual units, or ommatidia, results in a summation of like axes), the situation is different in rock lobsters, for they possess a 'fused' rhabdom characterised by centrally-connected rhabdomeres.

To determine the functional significance of the interweaving axons occurring in this type of compound eye, I traced the pathways of retinula cells and their axons in the eye of the rock lobster. Since Eguchi *et al.*³ have identified the locations of colour-sensitive cells in the similar retina of the crayfish, and Hafner⁴ has examined the fine-structural organisation of the lamina ganglionaris in the same animal, it also became necessary to know more about the region in between retina and lamina.

The rhabdom of *Panulirus longipes*, the Western Rock Lobster, is tiered and consists of seven large retinula cells. An eighth cell exists, but it is small and inconspicuous and apart from a tiny portion at the very distal end of the 'distal rhabdom' does not contribute to the rhabdom. As in *P. argus*⁵ the distal rhabdom consists of alternate layers of perpendicularly-oriented microvilli belonging to the rhabdomeres of the seven contributing retinula cells. One set of alternative layers is made

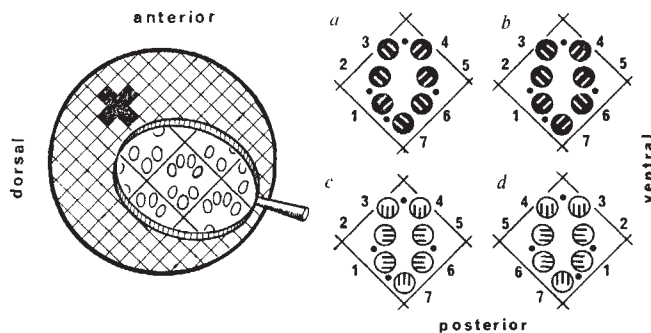


Fig. 1 Transverse section through the right compound eye of *P. longipes*. The seven retinula cells can be seen under a good binocular microscope at $\times 80$. Axons of one ommatidium interweave with those of four neighbouring ones so that a cross of five 'linked' facets results. *a-d*, Schematic representation of retinula cell positions and microvilli orientations of two superimposed layers of the distal rhabdom. The position of the four crystalline cone processes, indicated by small black circles, provides an excellent reference for identification of retinula cells, for example, cell 1 always lies between two processes. With regard to the interweaving pattern described in this paper and illustrated in Figs 2 and 3 arrangements (*a*)—modified after Eguchi and Waterman⁵—and (*b*) would result in bundles in which all axons originated from retinula cells with parallel microvilli. Most commonly found, however, were arrangements (*c*) and (*d*), which are mirror images and which give rise to the interweaving pattern shown in Fig. 3.

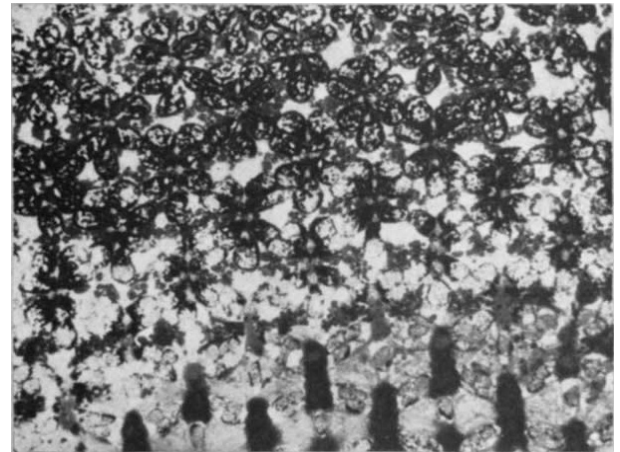


Fig. 2 Serial sections (2 μ m), stained with toluidine blue and examined under the light microscope, reveal how the regular interweaving pattern of retinula cell axons is produced. Erroneous combinations are extremely rare. Each retinula cell is approximately 15–18 μ m in diameter.

up by the microvilli of three retinula cells, the other set, which has its tubules at right angles to those of the three cells, comprises microvilli of the remaining four cells. This type of rhabdom, commonly called 'banded' because of its appearance in longitudinal sections, seems to be typical of decapod crustaceans⁶ and a few insects⁶, and is thought to be significant for *e*-vector discrimination^{7,8}. The proximal rhabdom in adult *P. longipes* (but not in larvae where it is also 'banded') differs from the thin cylindrical column of its distal counterpart in having a diameter which is ten times larger and microvilli which are not precisely oriented in two directions—as in the distal rhabdom—but run in all directions. In transverse section the proximal rhabdom resembles the leaf of a chestnut tree, in that there are seven large centrally-connected lobes.

The positions of the seven retinula cells are remarkably regular across the eye in each plane: in both left and right eye the seven retinula cells of each ommatidium are aligned diagonally so that four cells point anteriorly and three posteriorly (Fig. 1). The orientation of microvilli within these cells, however, may vary (Fig. 1*a-d*).

Serial sectioning revealed that some 50 μ m above the basement membrane interommatidial bundles of retinula cell axons are formed (Fig. 2). The axons from seven retinula cells of one ommatidium interweave with those of their four neighbouring facets in such a way that four bundles of three fibres, heterogeneous with regard to their ommatidial origin, plus one single axon are produced (Fig. 3). The single axon consistently joins one particular bundle (Fig. 3), a few microns below the basement membrane. To find which of the seven retinula cells, in terms of microvilli orientation, became combined in these interommatidial bundles, transverse sections at the level of the distal rhabdom (that is, where the villi were precisely oriented in two orthogonal directions) were examined in the electron microscope.

Once it was realised that the position of the narrow cone cell processes between the retinula cells was extremely consistent, identification of retinula cells became easy. The only cell with cone cell processes on both sides was called cell 1 and numbering of the remaining seven cells continued counter-clockwise.

In only very few cases was the arrangement of the seven cells similar to that reported by Eguchi and Waterman⁵ for *P. argus* (Fig. 1*a*). Equally rare (that is, less than 5%), was the arrangement shown in Fig. 1*b*. Had either of these two arrangements been realised more frequently, a surprisingly simple connectivity pattern of interconnections would have resulted from the particular way that retinula cell axons interweave before they penetrated the basement membrane: axons of cells with the same microvilli orientation would have been brought together. The most commonly observed configurations

were, however, retinula cells with microvilli arrangements as in Fig. 1c and d. The interweaving pattern of retinula cells in these cases would bring together the axons from cells which had their microvilli oriented at right angles to each other (Fig. 3).

There is evidence that the wiring pattern described is commonly found in decapod crustaceans with a 'banded rhabdom', but not in insects with a similar rhabdom⁹. For example, it has been reported¹⁰ that interweaving occurred in the eye of *Astacus* and that groups of three or four axons, derived from neighbouring ommatidia, penetrated the basement membrane. For the European lobster *Homarus* retinula fibres were reported¹¹ to "separate and end in different optic cartridges", but there was no further investigation.

Little is known so far of the functional significance of the described axonal arrangement but Horridge¹², who has correctly predicted interactions between retinula cells of one ommatidium with those of four neighbouring facets, believes

that this could make movement perception independent of colour change or polarisation plane.

Although these findings do not support Muller¹³, who believes that the frequently reported high polarisation sensitivity of individual retinula cells in crustaceans⁷ is partly achieved by electrical coupling between cells which are sensitive to the same orientation of the *e* vector of polarised light, they do not automatically lead to the conclusion that polarisation sensitivity will be lost during transmission to the central nervous system. Even with our wiring pattern, connections which would ensure that some information on polarised light would reach the brain, are possible.

Alternatively, however, it could be argued that a two-channel-analyser, such as the banded rhabdom, is a convenient way to achieve a polarisation-independent system if receptor cell outputs are summed¹⁴. Because polarisation patterns are much more prominent in the sea than in air¹⁵ (because of the dispersion of small particles in the water) it may be advantageous to a marine organism to be insensitive to them. The observed pattern of interweaving axons could be a direct consequence of this. The eighth retinula cell in the crab *Grapsus* with its microvilli arranged in two orthogonal directions¹⁶, the relatively poor responses from adult crabs to polarised light in behavioural experiments¹⁷, the failure to demonstrate an effect of polarised light on movement-sensitive fibres in the optic tracts of the crab *Podaphthalmus*¹⁸ and the tendency for the axons of retinula cells with microvilli oriented at right angles to be brought together in *P. longipes*, certainly support the latter interpretation.

This research was supported by a Queen's Fellowship in Marine Science. I thank Mr J. Kowarsky for reading the manuscript.

V. B. MEYER-ROCHOW

Department of Zoology,
University of Western Australia,
Nedlands, Perth, Western Australia 6009

Received August 12, 1974; revised February 4, 1975.

- ¹ Braitenberg, V. *Expl Brain Res.* 3, 271-298 (1967).
- ² Kirschfeld, K., *Expl Brain Res.* 3, 248-270 (1967).
- ³ Eguchi, E., Waterman, T. H., and Akiyama, J., *J. gen. Physiol.*, 62, 355-374 (1973).
- ⁴ Häfner, G. S., *J. comp. Neurol.*, 152, 255-280 (1973).
- ⁵ Eguchi, E., and Waterman, T. H., *Proc. int. Symp. Functional Org. Compound Eye*, 105-124 (Pergamon, Oxford, 1966).
- ⁶ Meyer-Rochow, V. B., *Cytobiology*, 4, 241-249 (1971).
- ⁷ Shaw, S. R., *Vision Res.*, 9, 999-1029 (1969).
- ⁸ Snyder, A. W., *J. comp. Physiol.*, 83, 331-360 (1973).
- ⁹ Meyer-Rochow, V. B., *J. Insect Physiol.*, 20, 573-589 (1974).
- ¹⁰ Parker, G. H., *Mitt. Stat. Zool. Napoli*, 12, 1-75 (1895).
- ¹¹ Hamori, J., and Horridge, G. A., *J. Cell Sci.*, 1, 249-256 (1966).
- ¹² Horridge, G. A., *Z. vergl. Physiol.*, 55, 207-224 (1967).
- ¹³ Muller, K. J., *J. Physiol., Lond.*, 232, 573-595 (1973).
- ¹⁴ Kirschfeld, K., *Z. Naturforsch.*, 27b, 578-579 (1972).
- ¹⁵ Waterman, T. H., *Science*, 120, 927-932 (1954).
- ¹⁶ Eguchi, E., and Waterman, T. H., *Z. Zellforsch.*, 137, 145-157 (1973).
- ¹⁷ Bäuerlein, R., *Forma et functio*, 1, 285-331 (1969).
- ¹⁸ Waterman, T. H., Wiersma, C. A. G., and Bush, B. M. H., *J. cell. comp. Physiol.*, 63, 135-155 (1964).

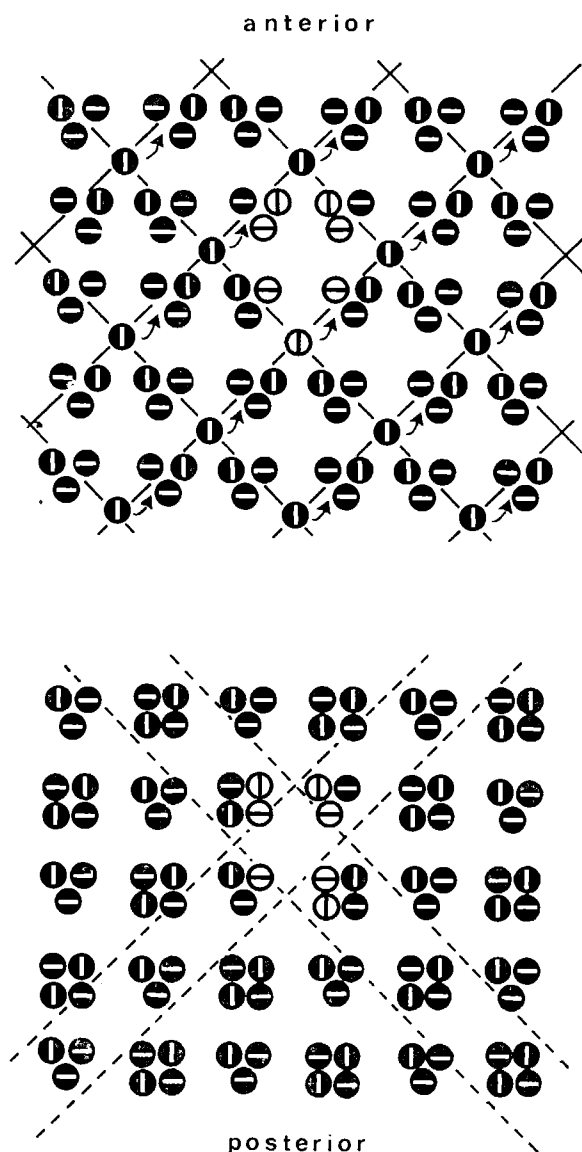


Fig. 3 Interweaving of retinula cell axons of one ommatidium (white circles) with those of four neighbouring facets results in four bundles of three fibres and one single axon (top). At 10 μ m below the basement membrane (bottom) the single axon joins the anterior bundle to the right (= ventral in the right eye), thus producing an array which may be envisaged as two sets of bundles: each set being composed entirely of three or four axon bundles in rows. As symbolised by the bar in each circle, unlike the bundles containing three fibres, each bundle of four consists of equal numbers of axons originating from cells with microvilli oriented at right angles to each other.

Membrane proteins related to water transport in human erythrocytes

CLASSICAL interpretations of the mechanism of water transport across mammalian red cell membranes assume the existence of aqueous membrane pores¹⁻³. As the permeability coefficient to water measured under an osmotic pressure gradient is usually significantly higher than the corresponding value measured under diffusional flow, the human red cell membrane is thought to act both as a selective solvent and a molecular sieve. Its ability to function as a molecular sieve depends on the existence of the pores, which could be assembled from aggregates of integral membrane proteins which span the membrane⁴. It is generally agreed that at least two proteins span the human red cell membrane⁵⁻⁷. Using polyacrylamide gel electrophoresis we have found that a band which contains one of these proteins is selectively labelled by a water-transport inhibitor.

We used 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), one of a group of sulphhydryl compounds⁸⁻¹⁰ that inhibit osmotic

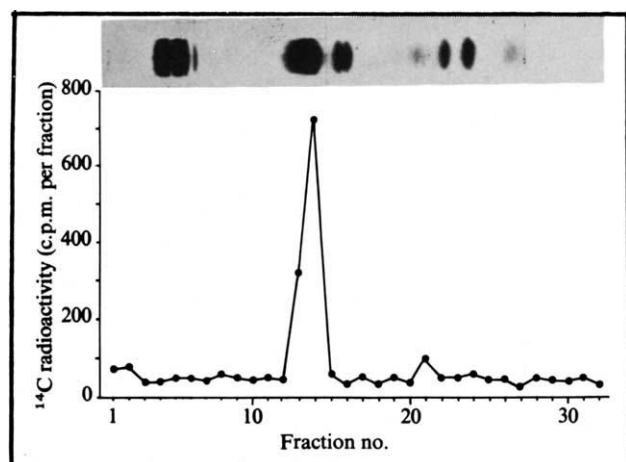
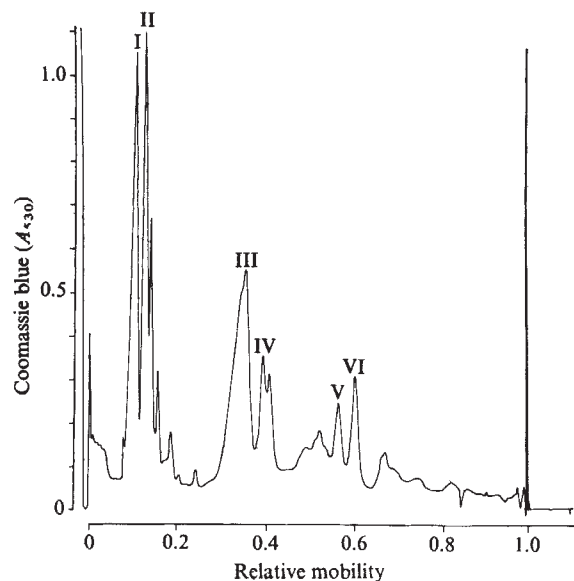


Fig. 1 Staining and labelling profiles of erythrocyte membrane proteins isolated from ^{14}C -DTNB-labelled cells. Ghosts isolated from red blood cells incubated with IAM, NEM and ^{14}C -DTNB were analysed for protein and radioactivity distribution by polyacrylamide SDS gel electrophoresis. The gels contained 5.6% acrylamide and 1% SDS and were prepared and stained by the method of Fairbanks *et al.*¹². Gels with radioactivity were sliced into 2-mm fractions, dissolved and analysed for distribution of ^{14}C -DTNB in a liquid scintillation counter. The amount of protein applied to each gel was 50 μg . Densitometry of gels is shown in Fig. 2. The identification of protein bands is according to the nomenclature of Fairbanks *et al.*¹².

water flow across some biological membranes. DTNB reacts with the smallest number of membrane thiol groups¹¹ (that is 10%, 2×10^{-17} mol per cell) of all the SH group reagents that inhibit water transport. Furthermore, it is specific for SH groups in that it only reacts by sulphhydryl-disulphide exchange. To minimise nonspecific binding of DTNB to membrane proteins we preincubated cells with N-ethylmaleimide (NEM) plus iodoacetamide (IAM) which react with 60% and 30%, respectively, of the membrane SH groups¹¹. These SH group reagents neither inhibit water permeability, nor do they affect the inhibitory action of DTNB⁸. NEM and IAM were also included in the cell lysing solution, as well as during the washing of the erythrocyte ghosts, so as to react with released SH-containing compounds which potentially could remove membrane-bound ^{14}C -DTNB.

Fig. 2 Densitometry of Coomassie blue stained gel in Fig. 1. Gels were scanned in a Gilford spectrophotometer with a model 2410 linear transport accessory at 530 nm (0.1 mm slit).



Fresh blood (25 ml) was mixed with 1 ml of 0.2M EDTA and an equal volume of cold 5 mM sodium phosphate-0.15 M NaCl (pH 7.4) and centrifuged at 1,000g for 10 min at 4 °C. Supernatant and buffy coat were removed and the packed cells were washed once and resuspended (30% haematocrit) in the buffered salt solution containing 1 mmol each of IAM and NEM. After incubation for 30 min at 37 °C, ^{14}C -DTNB (0.3 μmol) was added for a further hour, which is the time required for maximal inhibition of water transport. The cells were then collected by centrifugation and washed twice with the same incubation medium but without DTNB. Cell membranes were obtained by lysing 1.0–1.2-ml samples of the cell suspension in 40 ml of ice-cold 5 mM sodium phosphate solution, pH 8.0, containing 1 mM IAM and NEM. After centrifugation (20,000g for 30 min) the supernatant, which contained little radioactivity, was removed by aspiration. The ghost pellet was then obtained free from contaminating proteases in the tightly packed button, as described by Fairbanks *et al.*¹². Ghosts were washed three times in this way and finally dissolved by addition of an equal volume of a solution containing 2% sodium dodecyl sulphate (SDS),

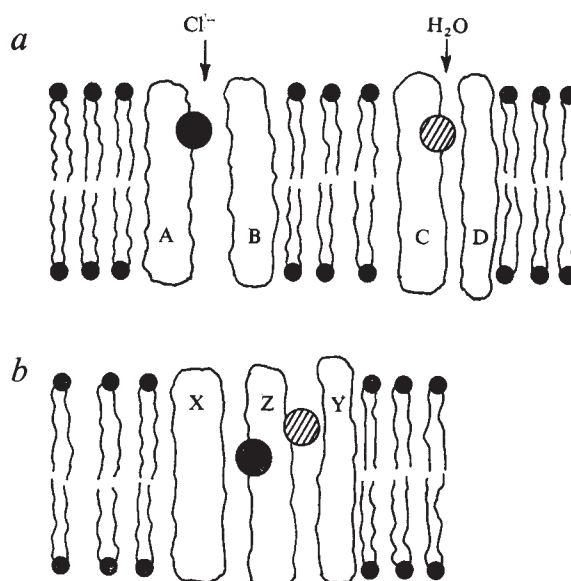


Fig. 3 *a*, Water and anion channels may be composed of entirely distinct polypeptide chains (although A may = B and C may = D) so that DIDS (filled circle) and DTNB (hatched circle) bind to separate polypeptide chains. *b*, Anion and water channels may be composed of several polypeptide chains, one of which is a common structural component of both (Z). DIDS and DTNB could react at different sites on Z thereby interfering with anion or water transport selectively.

20 mM Tris-HCl (pH 7.4) and 2mM EDTA. Aliquots of the dissolved membrane solution were then subjected to gel electrophoresis until the tracking dye ran 84 mm. The gels were stained with Coomassie blue, as described by Fairbanks *et al.*¹². Duplicate gels, unstained, were sliced and dissolved for liquid scintillation counting of ^{14}C . The gels were calibrated with chymotrypsinogen (25,000 daltons), ovalbumin (45,000 daltons) and γ -globulin (160,000 daltons) as molecular weight markers. The gel staining pattern and the distribution of radioactivity are shown in Fig. 1. Only one Coomassie blue-staining band was labelled with ^{14}C -DTNB. This was band III which comprised about 30% of the total membrane protein¹², and had a molecular weight of about 95,000 (ref. 13). Labelling was not observed if NEM and IAM were omitted during lysis or if dithioerythritol was added to lysed ghosts.

Gel densitometry was done using a Gilford spectrophotometer and model 2410 linear transport accessory, scanning at 530 nm (0.1 mm slit). The gel scans (Fig. 2) are essentially the same as those of Fairbanks *et al.*¹² except for the better resolution of the

two bands on the downward shoulder of peak II, and the resolution of peak IV into two bands. The latter was also observed by Fairbanks *et al.*¹² at lower concentrations of SDS. Figures 1 and 2 show that band III is quite dispersed and it may comprise more than one species of polypeptide¹⁴. This band has been reported to contain a phosphorylated intermediate of the $(\text{Na}^+ - \text{K}^+) - \text{ATPase}$ ^{15,16}. It also binds 4,4'-diisothiocyanato-2,2-ditriostilbene-disulphonate (DIDS), a specific inhibitor of anion permeability.¹³ The heterogeneity of protein band III is also indicated by the observation of Cabantchik and Rothstein¹⁴ that after Pronase treatment the staining of this band decreased and three individual bands were revealed. Based on studies involving proteolysis with chymotrypsin or pronase, Cabantchik and Rothstein¹⁴ concluded that ³H-DIDS was bound to a hydrophobic 65,000-dalton fragment of the 95,000-dalton protein.

Thus the labelling of band III by DIDS, an anion permeability inhibitor, and DTNB, a water permeability inhibitor, may result because each agent binds specifically to a different polypeptide chain. Another possibility is that the anion and water channels are each formed from a common structural polypeptide chain plus one or more polypeptide chains specific for the particular functional requirement (Fig. 3). DIDS and DTNB could conceivably react at different sites on the common structural polypeptide chain thereby impeding the function of only one type of channel in each case. We conclude from these experiments that polypeptide chains of molecular weight about 100,000 form part of the structure of aqueous channels which regulate non-carrier mediated transport in human erythrocyte membranes.

This work was supported by grants from the National Institutes of Health and the University of Connecticut Research Foundation.

PHILIP A. BROWN
MAURICE B. FEINSTEIN
RAMADAN I. SHA'AFI

University of Connecticut,
Schools of Medicine and Dental Medicine,
Farmington, Connecticut 06032

Received December 11, 1974; revised February 10, 1975.

- 1 Solomon, A. K., *J. gen. Physiol.*, **51**, 335 S (1968).
- 2 Sha'afi, R. I., Rich, C. T., Sidel, V. W., Bossert, W., and Solomon A. K., *J. gen. Physiol.*, **50**, 1377 (1967).
- 3 Sha'afi, R. I., and Gary-Bobo, C. M., *Prog. Biophys. molec. Biol.*, **26**, 105 (1973).
- 4 Singer, S. J., *A. Rev. Biochem.*, **44**, 866 (1974).
- 5 Bretscher, M. S., *Science*, **181**, 622 (1973).
- 6 Segrest, J. P., Kahane, I., Jackson, R. L., and Marchesi, V. T., *Archs biochem. Biophys.*, **155**, 167 (1973).
- 7 Steck, T. L., Fairbanks, G., and Wallach, D. F. H., *Biochemistry*, **10**, 2617 (1971).
- 8 Naccache, P., and Sha'afi, R. I., *J. Cell Physiol.*, **84**, 449 (1974).
- 9 Macey, R. I., and Farmer, R. E. L., *Biochim. biophys. Acta*, **211**, 104 (1970).
- 10 Macey, R. I., Karan, D. M., and Farmer, R. E. L., in *Passive Permeability of Cell Membrane* (edit. by Krenzer, F., and Segers, J. F. G.), 331, (Plenum, New York, 1972).
- 11 Rothstein, A., in *Topics in Membrane and Transport* (edit. by Bronner, F., and Kleinzeller, A.) **1**, 15 (1970).
- 12 Fairbanks, G., Steck, T. L., and Wallach, D. F. H., *Biochemistry*, **10**, 2606 (1971).
- 13 Cabantchik, Z. I., and Rothstein, A., *J. Membrane Biol.*, **15**, 207 (1974).
- 14 Cabantchik, Z. I., and Rothstein, A., *J. Membrane Biol.*, **15**, 227 (1974).
- 15 Avruch, J., and Fairbanks, G., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1216 (1972).
- 16 Knauf, P. A., Proverbio, F., and Hoffman, J. F., *J. gen. Physiol.* (in the press).

Abnormalities in membrane microviscosity and ion transport in genetic muscular dystrophy

We have demonstrated recently several functional differences between membrane-bound enzymes from tissues of muscular dystrophic chicks and of controls¹. Several other membrane anomalies have been associated with this disease²⁻⁷, but so far the only known biochemical anomaly in the affected membranes is a difference in lipid composition^{8,9}. This could cause all the observed functional defects, including those in enzyme activities and permeability, through an alteration in the membrane microenvironment¹⁰. Certain physical properties of the microenvironment can be studied by fluorescent polarisation techniques that measure the mobility of a lipid-soluble fluorescent dye^{11,12}. The basis for this approach is the fact that

membrane cholesterol, which strongly affects the microviscosity of liposomes¹⁰, is present in significantly greater than normal amounts in genetic muscular dystrophy. Using this approach, we found (Table 1) that the membranes of muscle, liver and erythrocytes of dystrophic chicks have a significantly higher microviscosity than normal controls. The greatest difference was in muscle, the site of the clinical symptoms of this genetic disease. The higher cholesterol:phospholipid ratio characteristic of the diseased membrane is sufficient to account for the observed increase in microviscosity¹¹.

Probably the most significant pathological expression of the defect of muscular dystrophy is an alteration in ion transport. It has been demonstrated that potassium efflux from dystrophic mouse muscle and human erythrocytes is significantly greater than normal^{13,14}. In view of the potential use of these observations in the screening and diagnosis of Duchenne muscular dystrophy (DMD), and in order to gain a deeper understanding of the defect we investigated the potassium and sodium transport properties of human erythrocytes. The results (Table 2) show that the potassium influx is significantly greater in both patients and carriers of the disease, relative to control. Ouabain, which inhibits the active component of the Na^+ and K^+ ion fluxes and the closely coupled ATPase activity¹⁵, inhibited (at 10^{-4} M) the potassium influx and the sodium efflux in the erythrocytes of all three groups. Thus the erythrocytes of DMD patients and carriers have a functional cation pump. On the other hand, Brown *et al.* showed that in the presence of Na^+ and K^+ ouabain enhanced the ATPase activity of erythrocytes from DMD patients (while it inhibited the ATPase activity of matched controls)¹⁶. The patients with DMD represent the first example of dissociation of the ouabain-inhibited component of sodium efflux and potassium influx from the ATPase activity. Together with the observation of the increased potassium efflux, this finding points to an imbalance between the active (pump) and passive (leak) ion movements in this disease.

The following tentative explanation relates the abnormality in ion fluxes to the observed increase in lipid microviscosity. The higher cholesterol: phospholipid ratio causes a tighter packing of lipid molecules resulting in an enhancement of the Van der Waals' interaction between adjacent lipid molecules¹⁷. These changes may create a defect in the polar pathways in the protein moiety of the membrane. This would increase the passive ion movement and cause an imbalance between the

Table 1 Membrane microviscosity in muscle sarcolemma, liver and erythrocyte plasma membranes of normal and dystrophic chicks

Tissue	Membrane microviscosity, $\bar{\eta}$, (cP)
Normal muscle	61 ± 8 (7)
Dystrophic muscle	117 ± 7 (14)*
Normal erythrocytes	365 ± 30 (6)
Dystrophic erythrocytes	435 ± 20 (14)*
Normal liver	153 ± 15 (14)
Dystrophic liver	234 ± 15 (19)*

*Significantly different from control at $P < 0.01$.

Muscle sarcolemma, liver and erythrocyte plasma membranes were prepared as before^{1,18-20} from the respective tissues of 20-d-old dystrophic chicks and age matched white leghorn (No. 25 Arbor Acre) control chicks. Steady-state fluorescence polarisation was measured with a Hitachi MPF-2A fluorescence spectrophotometer. Perylene at 10^{-6} M was used as the fluorescent probe. The dye was excited at 436 nm and emission was measured at 474 nm. Anisotropy of fluorescence was measured by polarising the exciting beam with a Polacoat IDSUV filter and observing the emission through the same type of filter. Fluorescence lifetimes were measured with a calibrated Ortec 9200-ns spectrometer. The calculation of the membrane microviscosity, $\bar{\eta}$, was based on the following relationship¹¹ $r_0/r = (1 + K/T\tau)/\bar{\eta}V(r)$ where r_0/r is the anisotropy τ is the mean fluorescence lifetime, K is Boltzman's constant, T is absolute temperature and $V(r)$ is the effective rotational molecular volume. Results represent the mean $\pm$ s.d. The number of determinations is given in parentheses.

Table 2 Potassium influx and sodium efflux in erythrocytes of DMD patients, carriers and controls (meq l⁻¹ erythrocytes × h)

Source of erythrocytes	Potassium influx		Sodium efflux	
	Total	+Ouabain	Total	+Ouabain
Normal subjects	1.90±0.05 (3)	0.35±0.03 (3)	3.6±0.5 (2)	1.4±0.3 (2)
Duchenne dystrophy patients	2.20±0.06 (4)*	0.43±0.03 (4)	3.5±0.4 (4)	0.9±0.1 (4)
Genetic carrier patients	2.25±0.10 (4)*	0.50±0.04 (4)		

*Significantly different from control at $P < 0.01$.

Blood was collected in heparinised syringes, centrifuged for 15 min at 1,500g. The plasma and buffy coat were removed carefully and the cells were washed three times in buffer as before²¹. Flux measurements were carried out following standard techniques^{21,22}. Results represent the mean and s.d. The number of determinations is given in parentheses.

active and passive ion transport. This study thus further supports the view that a lipid-related defect which affects the membrane microenvironment is the underlying genetic disorder of muscular dystrophy. The localisation of the pathological manifestation of the disease in the muscle is probably a reflection of the higher susceptibility of its specialised membrane system to this defect.

We thank Ms L. A. Bourret for technical assistance. This work was supported in part by grants from the Muscular Dystrophy Association of America, Inc. and the National Institutes of Health.

R. I. SHA'AFI
S. B. RODAN
R. L. HINTZ
S. M. FERNANDEZ
G. A. RODAN

University of Connecticut
Schools of Medicine and Dental Medicine
Farmington, Connecticut 06032

Received January 22; revised February 24, 1975.

- Rodan, S. B., Hintz, R. L., Sha'afi, R. I., and Rodan, G. A., *Nature*, **252**, 589 (1974).
- Sreter, F. A., Martonos, A., and Gergely, J., *Fedn Proc.*, **23**, 530 (1964).
- Matheson, D. W., and Howland, J. L., *Science*, **184**, 165 (1974).
- Morse, P. F., and Howland, J. L., *Nature*, **245**, 156 (1973).
- Murphy, D. L., Mendel, J. R., and Engel, W. K., *Archs Neurol.*, **28**, 239 (1973).
- Howland, J. L., and Challberg, M. D., *Biochem. biophys. Res. Commun.*, **50**, 574 (1973).
- Herzberg, G. R., Wallace, R., and Howland, J. L., *Biochem. biophys. Res. Commun.*, **55**, 551 (1973).
- Owens, K., and Hughes, B. P., *J. Lipid. Res.*, **11**, 486 (1970).
- Kunze, D., Reichmann, G., Egger, E., Leuschner, G., and Eckhardt, H., *Clin. Chim. Acta.*, **43**, 333 (1973).
- Papahadjopoulos, D., Cowden, M., and Kimelberg, H., *Biochim. biophys. Acta*, **330**, 8 (1973).
- Cogan, V., Shinitzky, M., Weber, G., and Mishida, T., *Biochemistry*, **12**, 521 (1973).
- Vanderkooi, J., Fischkoff, S., Chance, B., and Copper, R. A., *Biochemistry*, **13**, 1589 (1974).
- Lipicky, R. J., and Hess, J., *Am. J. Physiol.*, **226**, 592 (1974).
- Howland, J. L., *Nature*, **251**, 724 (1974).
- Tosteson, D. C., and Hoffman, F. F., *J. gen. Physiol.*, **44**, 169 (1960).
- Brown, H. D., Chattopadhyay, S. K., and Patel, A. B., *Science*, **157**, 1577 (1967).
- Demel, R. A., Kinsky, S. C., Kinsky, C. B., and Van Deenen, L. L. M., *Biochim. biophys. Acta*, **150**, 655 (1968).
- Severson, D. L., Drummond, G. I., Sulakhe, P. V., *J. biol. Chem.*, **247**, 2929 (1972).
- Neville, D. M., *Biochim. biophys. Acta*, **157**, 540 (1968).
- Bilezikian, J. P., and Aurbach, G. P., *J. biol. Chem.*, **249**, 5575 (1973).
- Sha'afi, R. I., and Lieb, W. R., *J. gen. Physiol.*, **50**, 1751 (1967).
- Dunham, B., and Hoffman, J. F., *J. gen. Physiol.*, **58**, 94 (1971).

Association of actin and myosin with secretory granule membranes

ACTIN and myosin have been found in many non-muscle cells¹. They are presumed to function in the generation of the force required in the various expressions of cytoplasmic or cell motility. Although both have been found in association with the plasma membrane²⁻⁵, nothing is known about the nature or consequences of this association. Equally uncertain is the mechanism of movement of secretory granules: synaptic vesicles, for example, are transported along neurones at high rates (up to 20 cm d⁻¹)⁶, probably in association with microtubules⁷. Roles for actomyosin in this process⁸ and in exocytosis⁹ have been proposed. We have used a new approach to the problem of actomyosin-membrane interaction, and report here the binding of actin

and myosin *in vitro* to a purified cytoplasmic membrane component.

Chromaffin granules, the catecholamine-containing vesicles of the adrenal medulla, are homologous in origin to the synaptic vesicles of sympathetic nerve cells. Both contain antigenically identical storage proteins¹⁰ and similar membrane enzymes¹¹. Bovine chromaffin granules are easily purified¹² and their membranes are released by lysis. We modified the conventional preparative methods by isolating the granules in 0.15 M KCl, buffered with 10 mM HEPES pH 7.0, and purifying them by centrifugation (40 min at 4 °C and 150,000g through 1.5 M sucrose in the same medium. Suspension of the pellet in buffered 0.15 M KCl leads to extensive lysis of the granules. The resulting mixture of membranes and partially-lysed granules was collected by centrifugation and used in subsequent experiments at a concentration of about 6 mg protein per ml. This membrane preparation resulted in some differences in minor protein bands on SDS polyacrylamide gel electrophoresis when compared with membranes prepared at low ionic strength¹².

The adrenal medulla contains both actin and myosin (ref. 13 and K. B. and J. H. P., unpublished). We have investigated the binding of smooth muscle myosin from chicken¹⁴ and of rabbit actin to chromaffin-granule membranes using the following method. Partially-lysed granules were incubated with the proteins under study (generally in 0.25 ml of 0.15 M KCl containing 10 mM HEPES and 1 mM MgCl₂ for 30 min at 24 °C); the suspension was then mixed with 0.5 ml 70% (w/w) sucrose in the same medium in a centrifuge tube (Spinco 2×0.5 inch), and overlaid with a linear gradient of sucrose (1.6 M to 0.6 M in the medium used for incubation). During the subsequent centrifugation (3 h at 4 °C and 200,000g), free proteins (storage proteins that have leaked from the damaged granules, together with test proteins that remain unbound) remain at the bottom of the tube, together with any unlysed granules; free membranes float up the tube to form a band at a position corresponding to their hydrated density (rather a broad band with a mean density of about 1.15 g ml⁻¹ for membranes prepared by this method). Ten fractions were collected from each gradient and the proteins in each fraction were analysed by electrophoresis in polyacrylamide slab gels containing sodium dodecyl sulphate¹⁵, followed by staining with Coomassie brilliant blue.

An analysis of granules incubated in the absence of other proteins is shown in Fig 1a. The major bands are chromogranins, the storage proteins found inside the granules. They are found in high concentration at the bottom of the gradient (fractions 1-3); the membranes, which are not obtained free of chromogranins, form a band in fractions 6-8. In some experiments the partially-lysed granules were subjected to freezing and thawing (Fig. 1c and d); in this case the membrane band is found further up the gradient, corresponding to a lower density.

If F-actin is incubated with the granules subsequent analysis of the gradient on SDS gels (Fig. 1b) shows that actin is distributed throughout the gradient; it is now a sig-

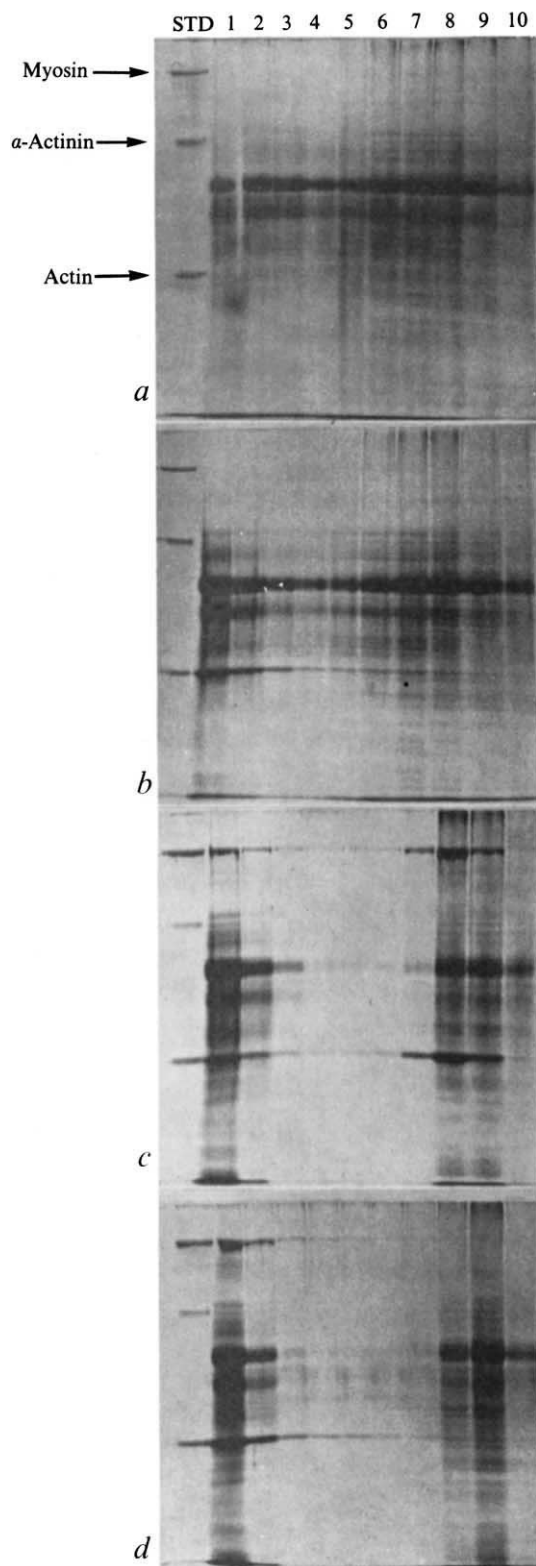


Fig. 1 SDS polyacrylamide slab gel electrophoresis showing fractions of sucrose gradients of chromaffin-granule 'ghost' membranes after incubation with test proteins. *a*, Membranes only; *b*, membranes and rabbit F-actin; *c*, membranes and F-actin and myosin (from chicken smooth muscle); *d*, as (*c*) but with 5 mM Mg-ATP during incubation and in the gradient. The left-hand column (STD) of each gel contained standard protein markers, myosin, α -actinin and actin; 1 represents the bottom fraction and 10 the top fraction of each gradient. Every fraction of a gradient is shown. The membranes in (*c*) and (*d*) had been frozen and thawed once and consequently having a lower density band higher up the gradients. Sucrose gradients were run as described in the text.

nificant component of the membrane fractions. This distribution is not affected by increasing the centrifugation time to 14 h. If ghosts prepared by the conventional low ionic strength procedure are used little actin is taken up the gradient. If F-actin is incubated in the absence of granule membranes it remains at the bottom of the gradient (no trace is found above fraction 3).

A very small amount of myosin can be shown to bind in the same way when myosin is incubated with membranes in the absence of actin; this binding is not affected by the method of preparation of the membranes. If, however, both actin and myosin are included in the incubation simultaneously, there is a great enhancement of the binding of each (Fig. 1*c*), presumably because of actin-myosin interaction on the membranes. This effect is abolished by the addition of excess (5 mM) Mg-ATP; inclusion of this in the incubation and the gradient reduces the binding of both proteins to the levels found in the absence of each other (Fig. 1*d*). These background levels of binding do not seem to be sensitive to Mg-ATP or to 2 mM Mg-pyrophosphate. Two out of twelve preparations of granules tested failed to bind actin to the extent shown in Fig. 1*b* although they continued to bind myosin; preliminary experiments suggest that this is caused by proteolytic action. Such preparations fail to show the enhancement effect with actin and myosin, suggesting that this enhancement depends on the successful binding of actin to the membranes.

We tried to assess the specificity of actin and myosin binding by incubating the membranes with other purified proteins and with a post-microsomal supernatant fraction from the adrenal medulla. In the first case, β -galactosidase, catalase and pyruvate kinase failed to bind, although a trace of phosphorylase A and tropomyosin binding was detected. Of the total supernatant proteins only three bound significantly, their mobilities on the gel corresponding to molecular weights of approximately 90,000, 70,000 and 45,000, the last probably being actin.

Fractions from the sucrose gradients were examined using electron microscopy. Filaments were frequently found to be associated with chromaffin-granule membranes when material from the main band of a gradient such as that shown in Fig. 1*b* was examined (Fig. 2); control gradients (Fig. 1*a*) contained no filaments. Many gradients were examined: a striking finding was that filaments generally terminated in a membrane (Fig. 2) and were rarely found to cross membranes (Table 1). Attachment of membranes to the centres of filaments might have been expected if membrane-bound myosin was involved.

The number of membrane 'ghosts' bearing filaments varies greatly from one experiment to another. The maximum

Table 1 Association of chromaffin-granule 'ghosts' with actin filaments

Experiment	Number of filaments counted			
	First	Second	Third	Total
Unassociated	113	34	86	233
Centrally-associated	26	11	24	61
Terminally-associated	99	30	48	177
Terminally-associated, but crossing an additional 'ghost'	14	1	2	17
Total number of filaments counted				488

Each experiment represents a different batch of chromaffin granules. Grids (see Fig. 2) were scanned at a magnification of 10,000 or 20,000, higher magnifications being used if necessary. Every filament found in a grid square was counted unless it could not be assigned unambiguously (for example, filaments crossing grid bars). 'Association' refers to apparent contact between a filament and an intact or fragmented membrane 'ghost' (see Fig. 2). Filaments which completely crossed, or which were tangential to membrane 'ghosts' were counted as 'centrally-associated'. The number of terminally-associated filaments which cross an additional 'ghost' is dependent on the frequency of 'ghosts' on the grid; this suggests that many 'centrally-associated' filaments are filaments with membranes inadvertently lying over them.

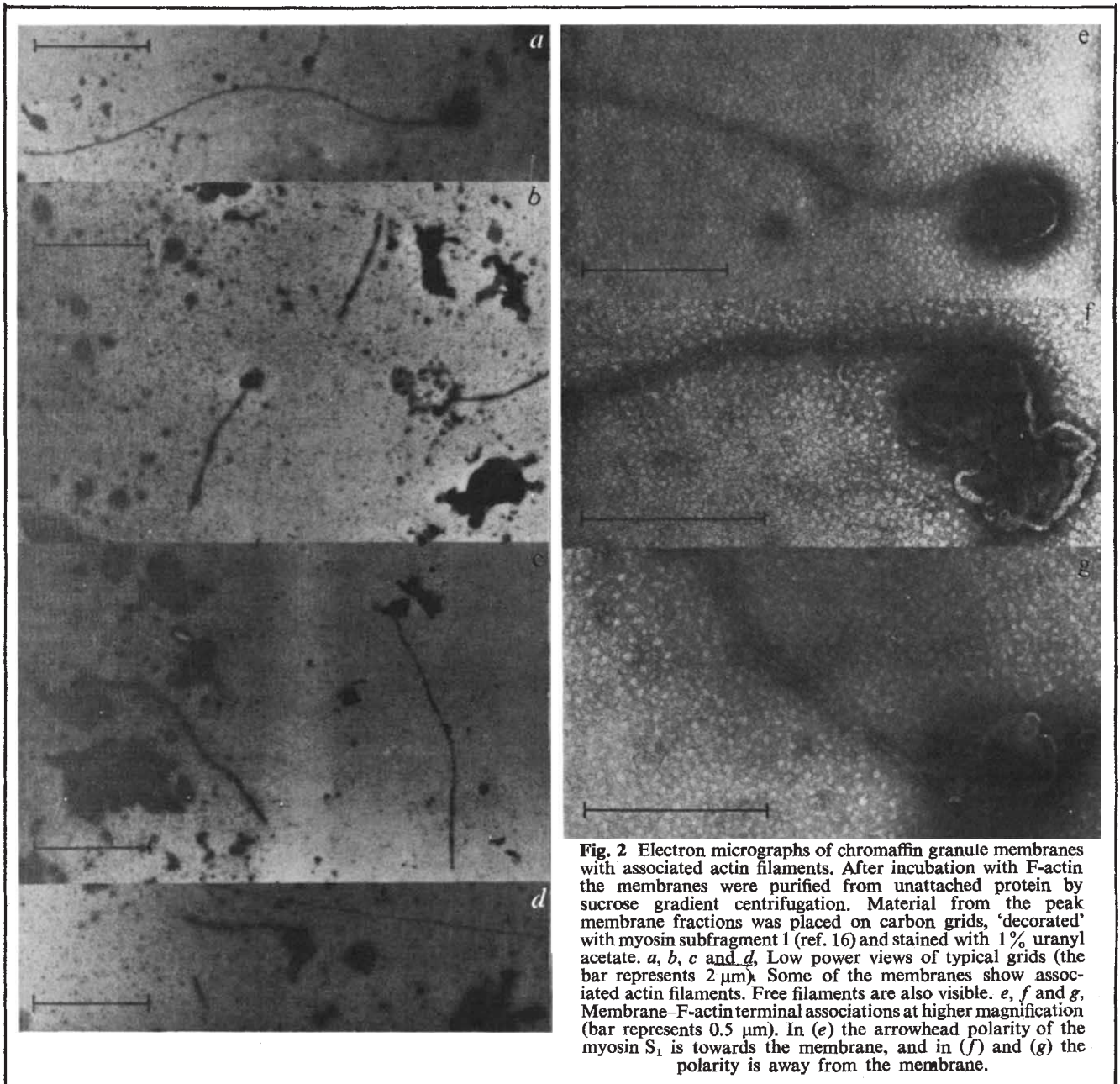


Fig. 2 Electron micrographs of chromaffin granule membranes with associated actin filaments. After incubation with F-actin the membranes were purified from unattached protein by sucrose gradient centrifugation. Material from the peak membrane fractions was placed on carbon grids, 'decorated' with myosin subfragment 1 (ref. 16) and stained with 1% uranyl acetate. *a, b, c* and *d*, Low power views of typical grids (the bar represents 2 μ m). Some of the membranes show associated actin filaments. Free filaments are also visible. *e, f* and *g*, Membrane-F-actin terminal associations at higher magnification (bar represents 0.5 μ m). In (*e*) the arrowhead polarity of the myosin S_1 is towards the membrane, and in (*f*) and (*g*) the polarity is away from the membrane.

number found has been about 10% per preparation. All preparations contained unattached filaments, however, even though specimens were taken from the main band on the gradient; such filaments were often short and may have arisen by fragmentation on the grid.

The attached actin filaments had both polarities as judged by their 'arrowhead' directions: 46 out of 68 examined had 'arrowheads' pointing away from the membrane. ('Decoration' of a filament attached to a muscle Z line shows 'arrowheads' pointing uniformly away from the attachment point¹⁶.) We did not find a convincing case of more than one filament attached to a 'ghost', although aggregates of 'ghosts' with several emerging filaments were sometimes observed.

Our experiments demonstrate that myosin and filamentous actin interact independently with secretory granule membranes. Because of the complexity of the present assay it has been difficult to investigate the specificity of these interactions and their optimal conditions, and we do not yet

know whether protein-protein interactions are involved. In view of the high frequency of interaction of membranes with the ends of actin filaments, it is attractive to speculate that there may be an actin-binding site in these membranes. Our finding that 'ghosts' prepared conventionally at low ionic strength bind very little actin is consistent with such a binding site being an α -actinin-like protein¹⁷, which is extracted at low ionic strength. Such a suggestion becomes more plausible now that one of us has identified a protein similar to α -actinin, in molecular weight and actin binding properties, from the particulate fractions of brain and fibroblasts (K. B., unpublished). If further work confirms a binding site, then the attachment of actin to these membranes could have a role in the transport of secretory granules from their site of formation in the Golgi apparatus to sites of exocytosis at the plasma membrane.

We thank Dr D. Goll for α -actinin, Dr J. Kendrick-Jones for F-actin and Dr A. Weeds for myosin S_1 ; Dr D. Bray for criticism and discussions; and Drs S. Hitchcock and M.

Stewart for helping us with the electron microscopy. K. B. acknowledges the support of an MRC scholarship.

K. BURRIDGE*
J. H. PHILLIPS†

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge CB2 2QH, UK

Received December 24, 1974.

* Present address: Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, New York 11724.

† Present address: Department of Biochemistry, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, UK.

- ¹ Pollard, T. D., and Weihing, R. R., *Critical Reviews in Biochemistry*, **2**, 1–65 (CRC, Cleveland, Ohio, 1974).
- ² Pollard, T. D., and Korn, E. D., *J. biol. Chem.*, **248**, 448–450 (1973).
- ³ Spudich, J. A., *J. biol. Chem.*, **249**, 6013–6020 (1974).
- ⁴ Kemp, R. B., Jones, B. M., and Gröschel-Stewart, U., *J. Cell Sci.*, **12**, 631–639 (1973).
- ⁵ Willingham, M. C., Ostlund, R. E., and Pastan, I., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4144–4148 (1974).
- ⁶ Dahlström, A., *Phil. Trans. R. Soc.*, **B261**, 325–358 (1971).
- ⁷ Heslop, J. P., *Soc. exp. Biol. Symp.*, **28**, 209–227 (1974).
- ⁸ Ochs, S., *Science*, **176**, 252–260 (1970).
- ⁹ Berl, S., Puszkun, S., and Nicklas, W. J., *Science*, **179**, 441–446 (1973).
- ¹⁰ Geffen, L. B., Livett, B. G., and Rush, R. A., *J. Physiol., Lond.*, **204**, 593–605 (1969).
- ¹¹ Flatmark, T., Lagercrantz, H., Terland, O., Helle, K. B., and Stjörne, L., *Biochim. biophys. Acta*, **245**, 249–252 (1971).
- ¹² Smith, A. D., and Winkler, H., *Biochem. J.*, **103**, 480–482 (1967).
- ¹³ Poisner, A. M., *Fedn Proc.*, **29**, 545 (1970).
- ¹⁴ Kendrick-Jones, J., *Phil. Trans. R. Soc.*, **B265**, 183–189 (1973).
- ¹⁵ Laemmli, U., *Nature*, **227**, 680–685 (1970).
- ¹⁶ Huxley, H. E., *J. molec. Biol.*, **7**, 281–308 (1963).
- ¹⁷ Goll, D. E., Suzuki, A., Temple, A., and Holmes, G. R., *J. molec. Biol.*, **67**, 469–488 (1972).

Table 1 Effect of oral thiamine on normal adult human hepatic branched chain α -ketoacid dehydrogenase

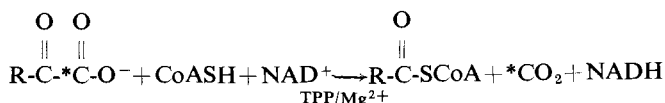
Substrate	Duration of 100 mg d ⁻¹ oral thiamine		
	0 d (6)	5–15 d (9)	18–28 d (3)
	(nmol CO ₂ per 15 min per mg protein)		
KIV	18.92 ± 5.34	19.84 ± 3.90	35.29 ± 6.96
KMV	9.53 ± 2.15	8.38 ± 2.24	12.79 ± 2.65
KIC	8.25 ± 2.73	6.88 ± 1.24	14.89 ± 2.61

$P < 0.001$

Assay conditions for CO₂ evolution are as follows: in 0.25 ml cofactors were present at a final concentration of 0.2 mM TPP, 0.3 mM MgCl₂, 0.2 mM CoA and 0.2 mM NAD⁺ in 50 mM Tris-HCl, pH 7.4 and 0.2 mM EDTA, and substrate at 2 mM. Mitochondrial protein was between 60 and 600 µg. ¹⁴CO₂ evolved, was trapped and quantitated as previously described¹. The number of patients in each time group is shown in parentheses. Values represent the mean and standard deviation of at least quadruplicate observations. *P* values were obtained using Student's *t* test and represent the probable difference between conditions after 5–15 d and 18–28 d for all three substrates.

were then suspended in MTE and mixed with an equal volume of MTE containing digitonin so that the final concentration was 1 mg digitonin per mg mitochondrial protein. Detergent action was stopped by addition of an equal volume of MTE after the reaction had proceeded for 20 min at 0 °C. Inner membranes were isolated immediately by centrifugation at 10,000g for 15 min and then suspended in an appropriate amount of MTE for use in the experiment.

Two methods of assay were used to follow dehydrogenase complex activity according to the products formed in the overall reaction:



Using 1-¹⁴C-labelled ketoacids as substrate, ¹⁴CO₂ produced was trapped in hyamine for quantitation by liquid scintillation. This assay was sensitive at pmol quantities of evolved product. The concentration of NADH was determined by spectrophotometry using an $\epsilon = 6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ at 340 nm under assay conditions as described in Table 1. Optical clarity was required for this assay. One mole of CO₂ was released for every mole of NAD⁺ reduced. In a typical experiment with 2 mM KIV as substrate, 13.82 nmol CO₂ per 15 min per mg were produced while the rate of NADH formation was 13.65 nmol per 15 min per mg.

Thiamine was administered orally (100 mg d⁻¹) to individuals with presumed normal branched chain dehydrogenase activity for up to 28 d before surgery, when open liver biopsy was performed for other diagnostic purposes. The dehydrogenase activity of mitochondrial inner membranes from these samples was measured and compared with activity in untreated controls.

Thiamine did not influence the branched chain α -ketoacid dehydrogenase activity until supraphysiological loading had been present for at least 18 d (Table 1). After this time, a 1.5–2.0-fold increase in specific activity was observed towards all three substrates.

There are several explanations for these results. First, thiamine could increase synthesis of these mitochondrial proteins. Second, thiamine or TPP could activate preformed inactive components of this complex. This is unlikely since time was required for the *in vivo* effect and *in vitro* addition of TPP to the assay media did not increase the specific activity. A third possibility is that thiamine or TPP at these supra-physiological concentrations maintains a conformation of the multienzyme complex which is less susceptible to degradation than is the naturally occurring holoenzyme. Such a mechanism has been postulated to explain the increase in hepatic pyridoxal-

Thiamine increases the specific activity of human liver branched chain α -ketoacid dehydrogenase

THIAMINE pyrophosphate (TPP), the active derivative of vitamin B₁, functions as a coenzyme in dehydrogenase reactions such as the oxidative decarboxylation of α -ketoisovaleric (KIV), α -keto- β -methylvaleric (KMV) and α -ketoisocaproic (KIC) acids in humans¹. Impairment of these reactions produces the group of disorders known as maple syrup urine disease^{2,3}. In the classic form of the disease, the ketoacids and their amino acid precursors accumulate in homozygous affected individuals and depress the function of the central nervous system early in life. Therapy involves restriction of branched chain amino acids in the diet to decrease their concentrations in the body fluids⁴. Further reduction in plasma leucine, isoleucine and valine has been achieved by supplementing this diet with thiamine for patients with partial (60%) and severe (95%) reduction in enzyme activity^{5,6}. Other investigators found no effect in patients lacking all enzyme activity^{7,8}. We are seeking an explanation for the clinical response at the enzyme level and have already reported that branched chain α -ketoacid dehydrogenase activity was increased in peripheral white blood cells. This occurred after 3 weeks of oral thiamine treatment in patients with 5% activity and in normal controls⁹. In contrast, activity in mitochondrial inner membranes from cultured normal and mutant skin fibroblasts was not stimulated directly by TPP/Mg²⁺, but was prolonged by the presence of TPP/Mg²⁺, suggesting that the cofactor increases the half life of the dehydrogenase complex. We have now found that thiamine supplementation of normal diets increases normal human liver branched chain α -ketoacid dehydrogenase activity.

Mitochondrial inner membranes were isolated from human liver obtained by open biopsy, and branched chain α -ketoacid dehydrogenase was measured before and during *in vivo* administration of 100 mg thiamine per day. Tissue was kept in ice cold 0.27 M mannitol buffered with 1.0 mM Tris-HCl, pH 7.3, and 0.1 mM EDTA (MTE). Tissues were assayed within 30 min after samples were taken. A decrease in mitochondrial dehydrogenase activity was noted if tissues were stored, cold or frozen. After homogenisation with a glass-Teflon tissue grinder, mitochondria were isolated by differential centrifugation and washed once with MTE. Mitochondria

5'-phosphate-dependent cystathionine synthase activity after prolonged administration of pyridoxine⁹.

This work was supported by a US Public Health Service research grant, a Clinical Research Center grant, and a research career development award (L.J.E.).

DEAN J. DANNER
EUGENE D. DAVIDSON
LOUIS J. ELSAS, II

Department of Pediatrics,
Division of Medical Genetics and Department of Surgery,
Emory University School of Medicine,
Atlanta, Georgia 30322

Received January 10; revised February 27, 1975.

1. Elsas, L. J., Priest, J. H., Wheeler, F. B., Danner, D. J., and Pask, B. A., *Metabolism*, 23, 569-579 (1974).
2. Dancis, J., Levitz, M., Miller, J., and Westall, R. G., *Br. Med. J.*, 1, 91-93 (1959).
3. Menkes, J. H., Hurst, P. L., and Craig, J. M., *Pediatrics*, 14, 462-466 (1954).
4. Dent, C. E., and Westall, R. G., *Archs Dis. Childh.*, 36, 259-268 (1961).
5. Elsas, L. J., and Danner, D. J., in *US-Japan Conference on Thiamine* (edit. by Gubler, C., and Fujiwara, M.), (Wiley Interscience, New York, in the press).
6. Scriver, C. R., Mackenzie, S., Clow, C. L., and Delvin, E., *Lancet*, i, 310-312 (1971).
7. Wong, P. W. K., Justice, P., Smith, G. F., and Hsia, D. Y. Y., *Clin. Genet.*, 3, 27-33 (1972).
8. Elsas, L. J., Pask, B. A., Wheeler, F. B., Perl, D. P., and Trusler, S., *Metabolism*, 21, 929-944 (1972).
9. Mudd, S. H., Edwards, W. A., Loeb, P. M., Brown, M. S., and Laster, L., *J. Clin. Invest.*, 49, 1762-1773 (1970).

Specific inhibition of ribosomal RNA synthesis *in vitro* by guanosine 3'-diphosphate, 5' diphosphate

CONSIDERABLE evidence has accumulated that guanosine 3'-diphosphate, 5'-diphosphate (ppGpp)¹ is involved in the regulation of ribosomal RNA (rRNA) synthesis in *Escherichia coli in vivo* (see ref. 2). The involvement of ppGpp is most convincingly indicated by results obtained under conditions of amino acid starvation. A strong increase in ppGpp accumulation is always accompanied by a concomitant decrease in stable RNA accumulation³. *In vitro*, a specific effect of ppGpp has not been found, neither in a purified⁴, nor in a crude system⁵. Travers *et al.*^{6,7} have proposed that ppGpp specifically affects rRNA synthesis by counteracting the effects of the protein elongation factor EF-Tu. Here, we show that ppGpp can specifically inhibit rRNA synthesis *in vitro* using purified RNA polymerase with two templates—DNA prepared by phenol extraction, and nucleoids prepared using methods developed by Pettijohn *et al.*⁸.

Table 1 shows that our hybridisation competition system has a very high specificity for rRNA, a low background and a high hybridisation efficiency. These characteristics together make it possible to detect clearly even small differences in rRNA synthesis.

Using nucleoids as template we found that $3.9 \pm 0.3\%$ (s.d.

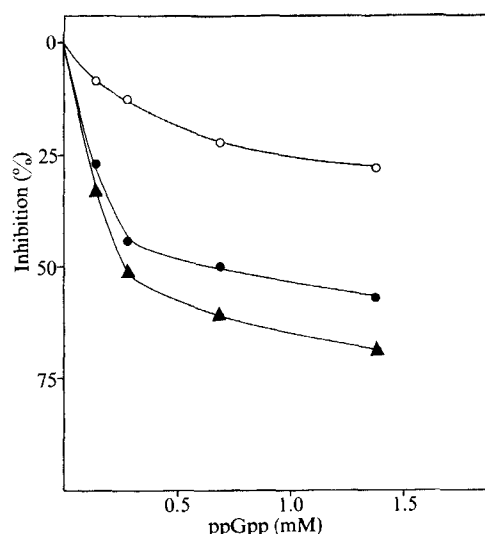


Fig. 1 Concentration dependency of ppGpp inhibition (results of experiments shown in Table 1). The percentage rRNA of total RNA has been calculated from the percentage ³H-UMP in rRNA by multiplication with 1.25 to correct for the base composition of rRNA relative to total DNA-complementary RNA. ○, Total RNA; ●, percentage rRNA; ▲, total rRNA.

mean) rRNA was made by added, purified RNA polymerase. Reaction conditions were as described in Table 1, except for the about tenfold less DNA concentration. ppGpp (0.6 mM) decreased total RNA synthesis by about 20% whereas rRNA was on the average 45% lower, resulting in a mean percentage rRNA synthesis of $2.7 \pm 0.4\%$. In four different experiments, the percentage rRNA was always lower when ppGpp was added.

The effect of ppGpp on total and rRNA synthesis was also assayed at various RNA polymerase-DNA ratios using phenol-extracted DNA. At all ratios tested ppGpp specifically inhibited rRNA synthesis (Table 2); the inhibition seems to be less pronounced at high and low ratios. The concentration dependency of ppGpp inhibition is shown in Fig. 1. Both total and ribosomal RNA synthesis are progressively inhibited by higher concentrations of ppGpp. Half maximal inhibition is obtained at about 0.15 mM which is the same as the apparent K_i for stable RNA accumulation *in vivo*¹². GDP (1 mM) did not decrease total or rRNA synthesis.

As we found in all conditions studied a specific effect of ppGpp on rRNA synthesis, that is, a stronger inhibition than that of total RNA synthesis, it is remarkable that others did not find this effect. Our hybridisation method is very sensitive and will allow detection of small differences, even at low rates of rRNA synthesis. In our case, inhibition of total RNA synthesis by ppGpp was less than that found by others^{4,5}. If inhibition of total RNA synthesis is large it could have 'drowned' a differential

Table 1 Hybridisation competition analysis of [³H]-RNA synthesised *in vitro*

ppGpp conc. (mM)	Input ³ H-UMP (d.p.m.) in RNA	-rRNA	³ H-UMP (d.p.m.) in hybrid +rRNA	Competed	³² P-rRNA (d.p.m.) in hybrid -rRNA	³² P-rRNA (d.p.m.) in hybrid +rRNA	³ H-UMP (d.p.m.) in rRNA	³ H-UMP in rRNA (%)
0.0	321,595	16,107	4,031	12,076	2,848	176	18,069	5.6
0.14	294,338	11,031	3,193	7,838	2,781	150	11,888	4.1
0.28	281,434	8,997	3,001	5,996	2,861	125	8,748	3.2
0.69	250,021	7,576	2,808	4,768	2,846	112	6,985	3.0
1.38	230,846	6,943	3,236	3,707	2,771	93	5,531	2.5

An aliquot (50 μ l) of *E. coli* DNA (44 μ g ml⁻¹) was added to 0.45 ml of a reaction mixture containing 40 mM Tris-HCl, pH 7.9 (25 °C); 0.4 mM potassium phosphate; 10 mM MgCl₂; 100 mM KCl; 0.3 mM each of ATP, CTP and GTP; 0.1 mM EDTA; 0.1 mM dithiothreitol and 0.07 mM ³H-UTP 1 Ci mmol⁻¹. The solution was incubated at 38 °C for 10 min, and then 32 μ g RNA polymerase was added. ppGpp was added just before RNA polymerase. After 30 min at 38 °C one-quarter volume of 1.5 M NaCl containing 0.15 M sodium citrate (pH 7) was added, and the solution was extracted once with the phenol mixture of Kirby⁹. Total RNA synthesis was measured in a 10 μ l sample to which 100 μ g yeast RNA was added as carrier by precipitation with 6% TCA containing 10 mM pyrophosphate. The remainder of the water layer was used directly in the hybridisation test. Hybridisation competition analysis was essentially according to Haseltine (ref. 4, Fig. 6), except that we used *Proteus vulgaris* DNA enriched for ribosomal DNA on a methylated albumin kieselguhr column instead of *E. coli* DNA. Values are the average of two triplicate hybridisations in which 20 μ l *in vitro* ³H-RNA and 4,005 d.p.m. ³²P-rRNA were used. ppGpp was prepared by the method of Cashel¹⁰. Further details of the experimental procedures were as described¹¹.

Table 2 Effect of ppGpp at various RNA polymerase to DNA ratios

Ratio of RNA polymerase to DNA (w/w)	Percentage rRNA		Percentage inhibition	
	-ppGpp	+ppGpp	total RNA	rRNA
1.7	3.4	2.4	7	34
14	8.5	4.4	16	56
28	5.5	2.5	12	60
43	4.3	2.4	16	53
57	2.4	1.5	12	45

The experiments were carried out as described in the legend to Table 1. The final concentration of DNA was $15 \mu\text{g ml}^{-1}$ at a ratio of 1.7, and $4.4 \mu\text{g ml}^{-1}$ at the other ratios. ppGpp was added at a final concentration of 0.6 mM .

effect on rRNA. Why inhibition of total RNA synthesis is lower in our system is unclear; our ppGpp preparation was checked for purity using polyethyleneimine thin layer chromatography (more than 90% pure).

The question arises how ppGpp effects its specific inhibition of rRNA synthesis. We have found that with the nucleoids packing of added RNA polymerase molecules on the ribosomal cistrons is maximal¹¹. Consequently, the elongation rate will determine the initiation frequency and it cannot be decided whether ppGpp acts specifically on elongation or initiation of rRNA. Further experiments are needed to clarify this point.

Whatever the mechanism, our results clearly show that ppGpp can specifically inhibit rRNA synthesis by purified RNA polymerase on a DNA template without the addition of other macromolecular components. Although extrapolation to the *in vivo* situation must be carried out with great caution our data support the hypothesis of a direct involvement of ppGpp in the (negative) control of rRNA synthesis.

We thank Miss Aly van Goor and Miss Jenny Brands for assistance. This work was carried out under the auspices of the Netherlands Foundation of Chemical Research (SON) with financial aid from the Netherlands Organisation for the Advancement of Pure Research (ZWO).

ALBERT J. J. VAN OUYEN
HERMAN A. DE BOER
GEERT AB
MAX GRUBER

Biochemisch Laboratorium,
Zernikelaan, Paddepoel,
Groningen, The Netherlands

Received December 31, 1974.

- ¹ Sy, J., and Lipmann, F., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 306-309 (1973).
- ² Lazzarini, R. A., and Johnson, L. D., *Nature new Biol.*, **243**, 17-19 (1973).
- ³ Cashel, M., and Callant, J., *Nature*, **221**, 838-841 (1969).
- ⁴ Haseltine, W. A., *Nature*, **235**, 329-333 (1972).
- ⁵ Murooka, Y., and Lazzarini, R. A., *J. biol. Chem.*, **248**, 6248-6250 (1973).
- ⁶ Travers, A., *Nature*, **244**, 15-17 (1973).
- ⁷ Travers, A., Kamen, R., and Cashel, M., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 415-418 (1970).
- ⁸ Pettijohn, D. E., Clarkson, K., Kossman, C. R., and Stonington, O. G., *J. molec. Biol.*, **52**, 281-300 (1970).
- ⁹ Kirby, K. S., in *Methods in Enzymology*, **12B** (edit. by Crossman, L., and Moldave, K.), 87-99 (Academic, New York, 1968).
- ¹⁰ Cashel, M., *Analyt. Biochem.*, **57**, 100-107 (1974).
- ¹¹ Van Ooyen, A. J. J., De Boer, H. A., Ab, G., and Gruber M., *Biochim. biophys. Acta* (in the press).
- ¹² Fiil, N. P., Von Meyenburg, K., and Friesen, J. D., *J. molec. Biol.*, **71**, 769-783 (1970).

Mouse L cells (American Type Culture Collection, Rockville) were seeded in 2-oz prescription bottles (Brockway Glass Co., Brockway, Pennsylvania) at a concentration of 4×10^5 cells ml^{-1} in 25 mM HEPES-buffered Medium 199 (ref. 2) with 10% decomplemented foetal calf serum. Ascorbate was added to a final concentration of 10^{-4} M or 10^{-5} M and the cultures were incubated for 18 h at 37°C . A dose of 10^{-3} M ascorbate was also used initially, but it usually made cultures acidic, with subsequent toxic effects. The medium was decontaminated, and the cell monolayer was exposed to synthetic poly(rI)·poly(rC) ($25 \mu\text{g ml}^{-1}$, Miles) and diethylaminoethyl-dextran ($100 \mu\text{g ml}^{-1}$) for 1 h. The cultures were washed three times with phosphate-buffered saline, refed with HEPES medium with included ascorbate and incubated at 37°C for another 10 h. The medium was then assayed by a colorimetric method³ to quantify cytopathic effects in L cell monolayers with vesicular stomatitis virus (VSV) as the challenge virus. Volumes of 1 ml of serial dilutions were used for assay, and the interferon titre, expressed in dye-uptake units ($\text{DU}_{50} \text{ ml}^{-1}$), was the reciprocal of the dilution which gave an absorbance reading at 540 nm midway between that of uninfected cell controls (100%) and the virus-infected controls (0%). Readings of duplicate dilutions generally agreed to within 10%. A standard reference interferon preparation (NIH reference interferon G002-904-511) with an assigned activity of 12,000 U ml^{-1} was included with each assay, and these reference titres are presented along with the experimental results. The NIH mouse reference interferon titres 22,500 U ml^{-1} as an average in repeated assays in our laboratory. Since the assigned

Table 1 Effect of ascorbate on interferon induction by poly(rI) poly(rC) in mouse L cell cultures

Concentration of ascorbate (M)	Interferon titres*		
	Experiment 1	Experiment 2	Experiment 3
None added	1,450	1,100	1,665
1.0×10^{-5}	1,785	ND	1,910
1.0×10^{-4}	3,330	3,105	5,060
NIH reference	20,185	15,300	28,545

*Titres are expressed in $\text{DU}_{50} \text{ ml}^{-1}$.
ND, not done.

potency of the reference interferon is 12,000 U ml^{-1} , conversion to reference units can be made by dividing the experimental values in the tables by the factor 1.9. The viral inhibitor was inactivated by trypsin, was non-toxic to L cells, did not directly inactivate VSV, and showed no antiviral activity in normal chick embryo fibroblasts.

The presence of ascorbate in cultures of L cells resulted in increased interferon production as demonstrated in three separate experiments. Values for 10^{-5} M and 10^{-4} M ascorbate (Table 1) show a dose response effect. Control cultures (no ascorbate added) produced a mean interferon titre of $1,405 \pm 285$. All three observations made with 10^{-4} M ascorbate exceeded this control value by more than three standard deviations and hence are considered significant.

It is well known that cells maintained in culture lose their resemblance to normal cells. In this connection, we have observed that primary and secondary cultures of mouse embryo fibroblasts are considerably less responsive to interferon induction by poly(rI)·poly(rC) than are cells of the mouse L line. Therefore, it seemed important to study the effects of ascorbate on cells newly established in culture. The results of two experiments (Table 2) with secondary cultures of normal mouse embryo fibroblasts revealed that, as in the case of the L cell line, 10^{-4} M ascorbate substantially increased the production of interferon.

Statistically, the interferon titres observed with 10^{-4} M ascorbate in both cases exceeded the mean value of 127.5 ± 46 for control titres by more than three standard deviations.

The role of ascorbate in potentiating the production of

Enhancement of interferon production by poly(rI)·poly(rC) in mouse cell cultures by ascorbic acid

WE have reported¹ an increased response to interferon induction in mice fed a diet containing vitamin C, and have now found a similar phenomenon *in vitro*. Addition of L-ascorbate to cultures of mouse cells (transformed L cells and normal embryonic fibroblasts) stimulated with polyinosinic acid · polycytidylic acid (poly(rI)·poly(rC)) resulted in increased synthesis of interferon.

Table 2 Effect of ascorbate on interferon induction by poly(rI)-poly(rC) in normal mouse embryo fibroblast cultures

Concentration of ascorbate (M)	Interferon titres*	
	Experiment 1	Experiment 2
None added	95	160
1.0×10^{-4}	310	415
NIH reference	11,600	26,635

*Titres are expressed in $\text{DU}_{50} \text{ U ml}^{-1}$.

interferon is not clear. Vitamin C may affect the interactions of poly(rI)-poly(rC) at the cell surface or within the cell⁴. Conceivably, ascorbate could function to increase the amount of interferon messenger RNA available for translation in cells stimulated with the polynucleotide by inhibition of a regulatory protein^{5,6} and promotion of the stability of the interferon messenger RNA⁷. There have been reports of stimulation of the antiviral activity of interferon by cyclic AMP⁸ and, more recently, by cyclic AMP and certain of its synthetic derivatives⁹. In this connection, preliminary experiments have suggested little difference in cyclic AMP levels between untreated and ascorbate-treated L cells as determined by radioimmunoassay¹⁰. Cultures assayed after 4 h of exposure to ascorbate contained 60, 74 and 60 pmol mg^{-1} nucleic acid, respectively, for cells untreated and treated with 10^{-5} and 10^{-4} M ascorbate. In another experiment, cells assayed after 18 h of treatment presented cyclic AMP levels of 75, 77 and 72 pmol mg^{-1} nucleic acid for the respective cultures. The participation of ascorbate in enhancing interferon synthesis in mouse cells *in vitro*, as well as in the intact animal¹, remains to be elucidated.

The technical assistance of David Gard and Sherrie Fitzgerald is acknowledged.

BENJAMIN V. SIEGEL

Department of Pathology,
University of Oregon Medical School,
Portland, Oregon 97201

Received December 20, 1974; revised February 7, 1975.

¹ Siegel, B. V., *Infect. Immun.*, **10**, 409-410 (1974).

² Eagle, H., *Science*, **174**, 500-503 (1971).

³ Finter, N. B., *J. gen. Virol.*, **5**, 419-427 (1969).

⁴ Burke, D. C., in *Interferons and interferon inducers* (edit. by Finter, N. B.), 107-133 (North Holland, Amsterdam, London, 1973).

⁵ Tan, Y. H., Armstrong, J. H., Ke, Y. H., and Ho, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 464-471 (1970).

⁶ Tomkins, G. M., Levinson, B. B., Baxter, J. D., and Dethlefsen, L., *Nature new Biol.*, **239**, 9-13 (1972).

⁷ Vilcek, J., and Havell, E. A., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3909-3913 (1973).

⁸ Friedman, R. M., and Pastan, I., *Biochem. biophys. Res. Commun.*, **36**, 735-740 (1969).

⁹ Allen, L. B., et al., *Proc. Soc. exp. Biol. Med.*, **146**, 580-584 (1974).

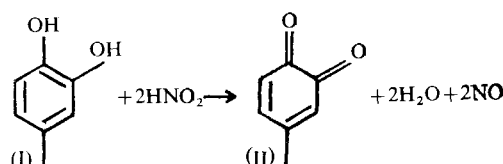
¹⁰ Steiner, A. L., Kipnis, D. M., Utiger, R., and Parker, C., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 367-373 (1969).

Possible cocarcinogenic effects of coffee constituents

ALTHOUGH the carcinogenic properties of secondary N-nitrosamines and related compounds are well established^{1,2}, their causal relationship to human cancer remains an open question because very little is known about the degree of human exposure to them. Carcinogenic N-nitrosamines have been detected in some nitrite-preserved foodstuffs, but only at relatively low levels^{3,4}, so the major uncertainty comes from their formation *in vivo* from ingested nitrite and amino compounds. Formation of N-nitrosamines in this way in the digestive tract of laboratory animals has been widely demonstrated (for typical examples, see refs 5-7) and has led to the induction of tumours characteristic of N-nitrosamines^{8,9}. We have suggested¹⁰ that the interaction between nitrite and phenolic materials should also be considered, because the latter are major dietary constituents which usually react much more rapidly than most amino compounds with nitrous acid. Certain natural phenols may therefore inhibit N-nitrosamine formation both in foodstuffs and in the digestive tract. We report here, however, that readily oxidised phenolic compounds act as catalysts rather than inhibitors for

N-nitrosamine formation from nitrite salts and secondary amines at gastric pH. This finding has important implications on the possible cocarcinogenic properties of several foodstuffs and beverages, including coffee.

Oxidation of *o*- and *p*-dihydroxyphenols (and of *o*- and *p*-aminophenols) to the corresponding quinones is well known¹¹ and may be anticipated for HNO_2 , itself an oxidising agent (standard oxidation potential at 298 K, $E^\circ = -0.98 \text{ V}$)¹². Our experiments confirm this expectation. Thus, treatment of 4-methylcatechol (I), for example, with dilute (10^{-2} - 10^{-3} M) HNO_2 at pH 2-4 and 25 °C gives rapid formation of the corresponding *o*-quinone (II) with evolution of nitric oxide (equation). Since the oxidation of 4-methylcatechol seems to be complete in about 8 min, it must be one of the best known scavengers for HNO_2 , but its presence, however, catalyses concurrent N-nitrosamine formation.



In the absence of added phenols, the more basic secondary aliphatic amines such as dimethylamine and piperidine react sluggishly with aqueous HNO_2 , even at pH 3-3.4, where the maximum rate is observed¹³; for example, $t_{1/2}$ is about 10h for dimethylamine with $[\text{HNO}_2] = 0.1 \text{ M}$ and 25 °C (ref. 13). N-nitrosamine formation is even slower at high pH and our finding for piperidine (Table 1), giving $t_{1/2}$ about 160 h at pH 4, $[\text{HNO}_2] = 0.1 \text{ M}$ and 25 °C, is typical. In the presence of readily oxidised phenols, however, formation of the N-nitrosamine is very much faster. Thus, typical results summarised in Table 1 show that addition of even 10^{-3} M 4-methylcatechol increases the rate of N-nitrosopiperidine formation by a factor of at least 1,000. In practice, significant amounts (about 10%) of N-nitrosopiperidine are found within the brief period (3 min) required to effect the first sampling, and its concentration reaches a maximum (Table 1) after about 10 min at 25 °C. Increasing the temperature to 39.3 °C has little effect on the amount of N-nitrosamine formed but its formation rate, as would be expected, is increased. Without the addition of 4-methylcatechol the amount of piperidine converted to the N-nitroso derivative after a reaction time of 10 min would be less than 0.1% even with the highest $[\text{HNO}_2]$ examined.

4-Methylcatechol bears a close structural resemblance to the phenolic component of chlorogenic acid (III), a very substantial (about 13% by weight), soluble constituent of coffee¹⁴. This compound (III) is therefore present in both 'instant' and ground coffee extracts to the extent of about 260 mg per cup¹⁴. Our experiments (Table 1) show that (I) and (III) exert very similar catalytic effects on N-nitrosamine formation. Thus addition of 10^{-2} M (III) leads to a maximum 56% N-nitrosopiperidine formation in about 10 min at pH 4 and 25 °C compared with 42% for 10^{-2} M (I). Further, we have found in preliminary experiments that a proprietary brand of 'instant' coffee, itself, when added to the buffered HNO_2 -piperidine reaction mixture at a realistic consumptive concentration of 2 g per 100 ml, also leads to rapid and substantial formation of N-nitrosamines.

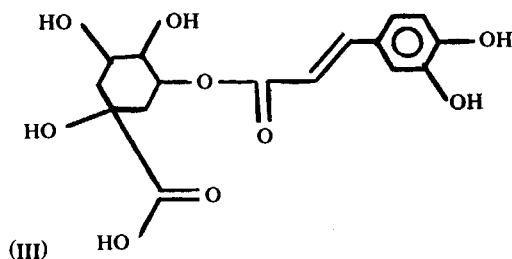


Table 1 Rates* of N-nitrosopiperidine formation in aqueous phthalate buffers at 25 °C.

pH	10 ² [NaNO ₂] M	[Added phenol] M	10 ⁶ k ₁ *s ⁻¹	% N-nitrosopiperidine† after t = 10 min‡
4.0	10	—	1.22	< 0.1
4.0	10	0.1 [I]	—	42
4.0	10	0.01 [I]	—	49
4.0	10	0.001 [I]	—	36
3.56	1.0	0.01 [I]	—	19
3.56	1.0	0.001 [I]	—	17.5
4.0	10	0.01 [III]	—	56.0
3.67	1.0	0.001 [I]	—	20§

* Rate = k₁ [Piperidine].

† Identified by ultraviolet, thin-layer chromatography and gas-liquid chromatography analyses.

‡ Significant N-nitrosopiperidine formed almost immediately and reaches a maximum in about 10 min (see text). The amount given is the percentage of the initial [piperidine] (10⁻³ M).

§ At 39.3 °C, where the maximum percentage reaction shown is reached after 3 min.

It is clear that both (I) and (III), under mildly acidic conditions, are very powerful catalysts for N-nitrosamine formation in aqueous media, and other readily oxidised phenols may be expected to act similarly. The mechanism of their catalytic action is the subject of current investigation, but we believe that either nitric oxide or an aryl nitrite is the very reactive nitrosating species involved. Significantly, oxidation of (I) and N-nitrosopiperidine formation both seem to be complete in about 8–10 min, and we have established that nitric oxide combines rapidly with piperidine under our experimental conditions.

The experimental conditions and reactant concentrations are not appreciably dissimilar from those expected for the human stomach following the ingestion of food containing 200 p.p.m. NaNO₂ (about 3 × 10⁻³ M) and a single cup of coffee (about 7 × 10⁻³ M in chlorogenic acid). The clear implication is that coffee, and other foodstuffs containing readily oxidised phenolic materials, may significantly increase human exposure to carcinogenic N-nitrosamines by catalysing their formation in the digestive tract.

We thank the SRC and the Cancer Research Campaign for their support.

B. C. CHALLIS
C. D. BARTLETT

Department of Organic Chemistry,
Imperial College, London SW7 2AZ, UK

Received January 15, 1975.

- Magee, P. N., and Barnes, J. M., *Adv. Cancer Res.*, **10**, 163 (1967).
- Druckrey, H., Preussman, R., Ivankovic, S., and Schmail, D., *Z. Krebsforsch.*, **69**, 103 (1967).
- Crosby, N. T., Foreman, J. K., Palframan, J. F., and Sawyer, R., *Nature*, **238**, 342 (1972).
- Panalakis, T., Iyengar, J. R., Donaldson, B. A., Miles, W. F., and Sen, N. P., *J. Ass. off. anal. Chem.*, **57**, 806 (1974).
- Mysliwy, T. S., Wick, E. L., Archer, M. C., Shank, R. C., and Newberne, P. M., *Br. J. Cancer*, **30**, 279 (1974) and references cited therein.
- Alam, B. S., Saporoschitz, J. B., and Epstein, S. S., *Nature*, **232**, 116 (1971).
- Sander, J., *Z. Physiol. Chem.*, **349**, 1691 (1968).
- Greenblatt, M., and Mirvish, S. S., *J. natn. Cancer Inst.*, **50**, 119 (1973); **46**, 1029 (1971).
- Sander, J., *Arzneimittel-Forsch.*, **21**, 1572, 1707 and 2034 (1971).
- Challis, B. C., *Nature*, **244**, 466 (1973).
- Bruce, J. M., *Rod's Chemistry of Carbon Compounds*, **3**, Part B, second ed. (edit. by Coffey, S.), 10 (Elsevier, Amsterdam, 1974).
- Charlot, G., Collumeau, A., and Marchon, M. J. C., *Selected Constants*, 32 (Butterworths, London, 1971).
- Mirvish, S. S., *J. natn. Cancer Inst.*, **44**, 633 (1970); *N-Nitroso Compounds: Analysis and Formation* (edit by Bogovski, E., Preussman, R., and Walker, E. H.), 104 (Int. Agency Res. Cancer, Lyon, 1972).
- Sivetz, M., *Coffee Processing Technology*, 166 (Air, Westport, 1963).

Tumour antigen specificity of a DNA-binding protein from cells infected with adenovirus 2

Two new polypeptides of molecular weights 75,000 and 45,000 are found in KB cells infected with human adenovirus type 2 (Ad 2), (ref 1). They represent the major labelled protein components of a nuclear membrane fraction ('DNA replication complex') that is capable of synthesising adenovirus DNA *in vitro*¹⁻³. It has been proposed that these polypeptides are early viral gene products, for they are synthesised soon after infection in the presence of 1-β-D-arabinosylcytosine (Ara C), an inhibitor of DNA synthesis¹. Their association with the DNA replication

complex suggested that they may have an affinity for DNA and could play a role in the replication of adenovirus DNA. It was subsequently shown that these two polypeptides bind strongly to single-stranded DNA-cellulose columns and that large amounts of these 'DNA-binding proteins' can be isolated from the cytoplasm of the infected cells³. Two polypeptides of similar molecular weights (72,000 and 48,000) were independently isolated from cell extracts of monkey kidney cells, abortively infected with Ad 5, by their ability to bind to single-stranded DNA-cellulose^{4,24}. The use of temperature-sensitive mutants of Ad 5 indicated that these polypeptides are viral-coded^{4,24}. Comparison of tryptic maps suggested that the 48,000 component was a degradation product of the 72,000 polypeptide (A. J. Levine, unpublished). A polypeptide of molecular weight about 65,000–75,000 has also been detected by polyacrylamide gel analysis in KB cells soon after infection by Ad 2 and Ad 5 (refs 5–7) and in HeLa cells infected by Ad 2 (ref. 8).

Ad 2 and Ad 5 (together with Ad 1 and Ad 6) comprise the group C human adenoviruses⁹. Members of this group transform rat cells *in vitro*¹⁰, and DNA fragments of subgenomic size isolated from Ad 2 and Ad 5 have been reported to transform hamster, rat and human cells *in vitro*¹¹. Most transformed rat cell lines derived by infection with Ad 2 contain DNA sequences representing about 14% of the viral genome and contain tumour (T) antigens¹². T antigens are virus-specific early proteins that are synthesised during productive infection as well as in transformed and tumour cells⁹. They are generally detected by two immunological methods, complement fixation (CF) of cell extracts and immunofluorescence of fixed cells, using sera from hamsters bearing adenovirus-induced tumours. T antigens induced by adenoviruses within the same group cross-react serologically⁹. We describe below the results of three types of immunological measurements—CF analysis, radioimmune precipitation, and radioimmune precipitation-inhibition—which show that the DNA-binding protein of molecular weight 75,000 (75k protein) in cells infected with Ad 2 has T-antigen specificity.

The 75k protein was purified from the cytoplasm of infected cells by chromatography on a single-stranded DNA-cellulose column as described³. The bulk of the 75k protein is present in the fraction eluting at 0.6 M NaCl from the DNA-cellulose column (0.6 M eluate). By modifying our published procedure³ (Fig. 1), we obtained preparations of 75k protein free of the 45,000 molecular weight protein. Analysis of polypeptides labelled with ³H-leucine in the 0.6 M eluate by polyacrylamide gel electrophoresis revealed a single major polypeptide peak of a molecular weight of 75,000 with only minor amounts of contaminating labelled polypeptides (Fig. 1). Unlabelled 0.6 M eluates were also electrophoresed and shown to contain 75k protein as the predominant constituent after staining with Coomassie brilliant blue.

Unlabelled 0.6 M eluates were assayed by a CF microassay using hamster sera against several different T antigens (anti-T sera). The anti-Ad 1 and Ad 2 T sera and ascitic fluid were prepared in hamsters by injecting extracts of transplanted hamster tumours induced by Ad 1-SV40 or Ad 2-SV40 hybrid viruses. Anti-Ad 12 (group A) and anti-SV40 T sera were

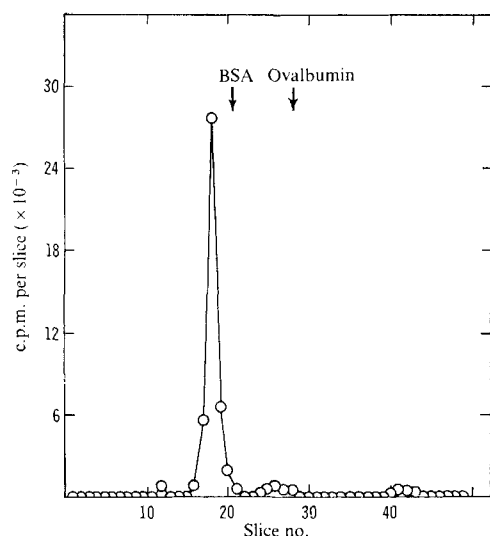


Fig. 1 Gel electrophoresis of DNA-binding proteins in the 0.6 M eluate from a single-stranded DNA-cellulose column. KB cells were grown in suspension in Eagle's MEM containing 5% horse serum and were infected with Ad 2 at a multiplicity of 100 PFU per cell². At 2 h after infection, 25 $\mu\text{g ml}^{-1}$ of Ara C were added. At 6 h, cells were centrifuged, washed with leucine-free MEM at 37 °C, and resuspended in leucine-free MEM with 5% horse serum. Ara C (25 $\mu\text{g ml}^{-1}$) and ³H-leucine (2 $\mu\text{Ci ml}^{-1}$, 41 Ci mmol⁻¹) were added and the cells were further incubated until 24 h after infection. Cells were harvested by centrifugation, washed with phosphate-buffered saline, and resuspended in 0.01 M Tris, pH 7.4, 0.01 M NaCl, 0.0015 M MgCl₂ at a density of 10⁷ cells ml⁻¹. After standing in ice for 15 min, the swollen cells were disrupted by 15–20 strokes of a tight-fitting Dounce homogeniser and nuclei were removed by centrifugation at 2,000g for 10 min through a 5 ml cushion of 25% sucrose in 0.01 M Tris, pH 7.4, 0.01 M NaCl, 0.0015 M MgCl₂. The supernatant cytoplasmic fraction was treated with 20 $\mu\text{g ml}^{-1}$ of DNase I for 30 min at 22 °C in the presence of 0.05 M MgCl₂ and 0.001 M 2-mercaptoethanol. The treated cytoplasm was dialysed overnight at 4 °C against at least 100 volumes of dialysis buffer containing 0.02 M Tris, pH 8.1, 10% glycerol, 0.05 M NaCl, 0.005 M EDTA and 0.001 M 2-mercaptoethanol. A precipitate formed, which was removed by centrifugation before DNA-cellulose chromatography. Single-stranded DNA-cellulose was prepared according to Litman¹³. All DNA-cellulose column procedures were performed at room temperature. Dried single-stranded DNA-cellulose (0.4 g) was suspended in dialysis buffer and used to pack a 1.8 cm³ column (0.8 × 3.0 cm). The column was washed with 10 ml of dialysis buffer and loaded with the cytoplasmic extract (approximately 24 mg protein) at a flow rate of 10 ml h⁻¹. The column was washed with 10 ml of dialysis buffer and eluted with 10 ml each of dialysis buffer containing 0.4 M NaCl and 0.6 M NaCl. Bovine serum albumin (50 $\mu\text{g ml}^{-1}$) was added to the 0.6 M eluate to stabilise the DNA-binding proteins. The 0.6 M eluate was concentrated by dialysis against 0.02 M Tris, pH 8.1, 0.05 M NaCl, 60% glycerol, 0.001 M EDTA and 0.001 M 2-mercaptoethanol overnight at 4 °C and was stored at -20 °C. SDS gel electrophoresis was performed¹⁴ with gels containing 6% polyacrylamide and 0.16% bis-acrylamide at 5 mA per gel for 30 min, then at 10 mA for an additional 6–8 h. The gels were fractionated into 2 mm slices with a Gilson gel fractionator and counted in Aquasol (New England Nuclear). Marker bovine serum albumin and ovalbumin were electrophoresed in parallel gels and visualised by staining with Coomassie brilliant blue.

obtained from hamsters bearing tumours induced by those viruses. Typical results presented in Table 1 show positive fixation to a titre of 1:32 with anti-Ad 1 and Ad 2 T sera. Anti-Ad 12 T serum, anti-SV40 T serum, and goat antiserum to purified Ad 2 virions were all negative. Control preparations consisting of disrupted Ad 2 virions and a 0.6 M NaCl eluate from DNA cellulose chromatography of cytoplasm from mock-infected cells (Table 1, mock 0.6 M eluate) did not react with the anti-T sera. These results indicate that the 75k protein is a non-virion, group C-specific T antigen found only in infected cells.

CF titres of the 0.6 M eluate with anti-Ad 1 and Ad 2 T sera were relatively low and could perhaps reflect the assay of a minor contaminant rather than the 75k protein. We therefore tested

labelled 0.6 M eluates in an indirect radioimmune precipitation assay modified from Horwitz and Scharff¹⁸. Immunoglobulins isolated from the hamster anti-T serum and ascitic fluid and goat anti-virion serum were used rather than whole sera to facilitate comparison between different sera. Since over 90% of the radioactivity in the 0.6 M eluate is present in the 75k protein, immunoprecipitation of more than 10% of the radioactivity would mean that the 75k protein and not a minor contaminant is specifically measured. Two different labelled 0.6 M eluates were assayed, and the results of a typical experiment are presented in Fig. 2. A maximum of 63–66% of the input radioactivity in the 0.6 M eluate was specifically immunoprecipitated with anti-Ad 1 and anti-Ad 2 immunoglobulin, whereas only 7–10% (same value as the normal immunoglobulin control) was precipitated by the immunoglobulins from the anti-SV40 T or goat anti-virion sera. The lack of quantitative precipitation could be caused by labelled contaminants in the 0.6 M eluate; or by a major second protein comigrating with the 75k protein (two early viral mRNA molecules of nearly identical size that could code for the 75k protein were recently reported²⁰); or by the presence

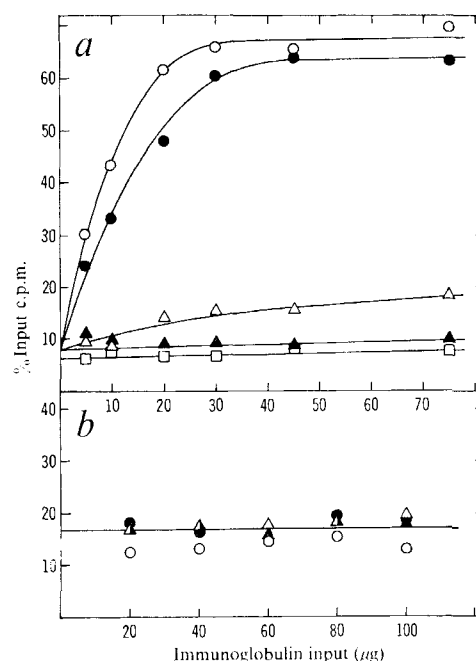


Fig. 2 Radioimmune precipitation assay of 0.6 M eluates from Ad 2-infected and mock-infected cells for Ad 2 T antigen. Immunoglobulins were isolated from hamster anti-T sera and ascitic fluid and from goat antiserum to Ad 2 virions by DEAE-cellulose chromatography and concentrated by precipitation with 50% saturated (NH₄)₂SO₄ (ref. 19). The immunoglobulins were dialysed against PBS-EDTA (0.01 sodium phosphate, pH 7.2, 0.1 M NaCl and 0.005 M EDTA). The 0.6 M eluates were diluted in PBS-EDTA containing 1% desoxycholate and 1% Triton X-100 and clarified at 12 000g for 15 min before assay. Constant amounts of labelled eluate were added to increasing amounts of the hamster or goat immunoglobulin in 300 μl PBS-EDTA containing 1% desoxycholate and 1% Triton X-100. Immunoglobulins from a pool of normal hamster sera or a normal goat serum were added to each tube to provide a total of 125 μg of protein to maintain a uniform precipitate in all tubes. Control tubes containing only normal hamster or goat immunoglobulin were used to determine the background level of nonspecific precipitation. The mixtures were incubated at 37 °C for 30 min in siliconised 2 ml centrifuge tubes (Kimax) and 50 μl goat anti-hamster IgG or 60 μl pig anti-goat IgG sera were added (optimal proportions were predetermined in precipitation assays). The tubes were further incubated for 2 h at 37 °C and the precipitated complexes were sedimented at 900g for 10 min. The precipitates were dispersed by mixing (Vortex), washed twice in PBS-EDTA containing 1% of desoxycholate and 1% Triton X-100, dissolved in 0.25 M acetic acid, and counted in Aquasol. *a*, 0.6 M eluate from infected cells. Input, 8,522 c.p.m., 10,000 c.p.m. μg^{-1} . *b*, Mock 0.6 M eluate. Input, 4,834 c.p.m., 550 c.p.m. μg^{-1} . ○, Anti-Ad 1 T; ●, anti-Ad 2 T; △, anti-Ad 12 T; ▲, anti-SV40 T; □, goat anti-Ad 2 virions.

Table 1 Complement fixation assay of the Ad 2 75k DNA-binding protein

Source of antigen	Hamster anti-T sera against:				Goat antiserum against Ad 2 virions
	Ad 1	Ad 2*	Ad 12	SV40	
Ad 2 0.6 M eluate	1 : 32†	1 : 32	≤ 1 : 2‡	≤ 1 : 2	≤ 1 : 2
Mock 0.6 M eluate	≤ 1 : 2	≤ 1 : 2	≤ 1 : 2	≤ 1 : 2	ND
Disrupted Ad 2 virions	≤ 1 : 2	≤ 1 : 2	≤ 1 : 2	≤ 1 : 2	1 : 256

The cytoplasm from cells infected with Ad 2 or mock-infected cells (treated in the same manner as infected cells but no virus was added) was fractionated on single-stranded DNA cellulose columns as described in Fig. 1. Ad 2 virions purified as described¹⁶ using CsCl for RbCl in the density gradient step were disrupted by the method of Prage *et al.*¹⁶ and sonicated before assay since the released cores tended to aggregate. The 0.6 M eluates and the preparation of disrupted Ad 2 virions were assayed by the CF microassay¹⁷. Pre-titrated hamster and goat antisera were heated at 56 °C for 30 min and used at a dilution that gave five to ten units of antibody. Assays were performed with 1.5 units of complement. Similar results were obtained with a second preparation of 0.6 M eluate.

* Ascitic fluid rather than serum was used.

† Titre expressed as the highest dilution of antigen giving complete fixation.

‡ The titre is 1 : 2 or lower; 1 : 2 is the anticomplementary titre of the antigens alone and represents the lower limit of the assay. ND, not done.

of some denatured 75k protein that does not react with anti-T serum. Some precipitation above background level was consistently observed with the anti-Ad 12 T immunoglobulin. This could indicate that a low degree of cross-reactivity between T antigens of group A and group C is detected by the sensitive radioimmune precipitation assay. A mock-infected 0.6 M eluate (labelled with a fivefold higher level of ³H-leucine since few host proteins are present in the 0.6 M eluate) was also assayed (Fig. 2). This preparation did not react above the background level observed with normal immunoglobulin.

The specificity of the radioimmune assay for T-antigen activity was demonstrated by radioimmune-inhibition experi-

ments. As shown in Fig. 3, the precipitation of ³H-labelled protein from the 0.6 M eluate with a saturating level of anti-Ad 2 T immunoglobulin could be inhibited by 80% by preincubating the immunoglobulin with increasing amounts of unlabelled 0.6 M eluate or unlabelled cytoplasm from infected cells. No inhibition was observed on preincubation with disrupted Ad 2 virions, or with the cytoplasm from mock-infected cells. Note that a twentyfold higher input of infected cytoplasm was needed to obtain the same level of inhibition observed with the 0.6 M eluate. This result indicates the feasibility of measuring 75k protein in unlabelled extracts of infected, transformed, or tumour cells by radioimmune-inhibition assay.

No function has yet been assigned to the 75k protein. A protein that binds to single-stranded DNA is required for the replication of bacteriophage T4 (ref 21). We have shown that the 75k DNA-binding protein fulfils the criteria for T antigen, as it reacts with antibody elicited to Ad 1 and Ad 2-transformed cells in hamsters. It seems likely that there is more than one T antigen in Ad 2-transformed cells since several virus-specific RNA species have been detected²². Carroll *et al.*²³, have recently shown that a T antigen isolated from SV-40-transformed mouse cells binds to double, but not to single-stranded DNA. It will be of interest to determine whether all Ad 2-transformed cell lines and tumours, as well as the rat line recently transformed by a 1.6 × 10⁶ molecular weight fragment of Ad 2 and Ad 5 DNA (ref 11), synthesise the 75k protein. This may indicate whether the 75k protein has an obligatory role in the induction and maintenance of the transformed state.

We are grateful to Dr R. Gilden, Flow Laboratories, and the program of Resources and Logistics, National Cancer Institute for gifts of antisera. This work was supported by a Public Health Service Grant from the National Institutes of Allergy and Infectious Diseases and by the Virus Cancer Program of the National Cancer Institute, NIH. M.G. is a Research Career Awardee of the NIH.

Z. GILEAD
M. Q. ARENS
S. BHADURI
G. SHANMUGAM
M. GREEN

*Institute for Molecular Virology,
St Louis University School of Medicine,
3681 Park Avenue,
St Louis, Missouri 63110*

Received January 6, 1975.

- Yamashita, T., and Green, M., *J. Virol.*, **14**, 412-420 (1974).
- Shanmugam, S., Bhaduri, S., Arens, M. Q., and Green, M., *Biochemistry*, **14**, 332-337 (1975).
- Yamashita, T., Arens, M. Q., and Green, M., *J. biol. Chem.* (in the press).
- van der Vliet, P. C., and Levine, A. J., *Nature new Biol.*, **246**, 170-174 (1973).
- Russel, W. C., and Skehel, J. J., *J. gen. Virol.*, **15**, 45-57 (1972).
- Anderson, C. W., Baum, P. R., and Gesteland, R. F., *J. Virol.*, **12**, 241-252 (1973).
- Bablian, R., and Russel, W. C., *J. gen. Virol.*, **24**, 261-279 (1974).

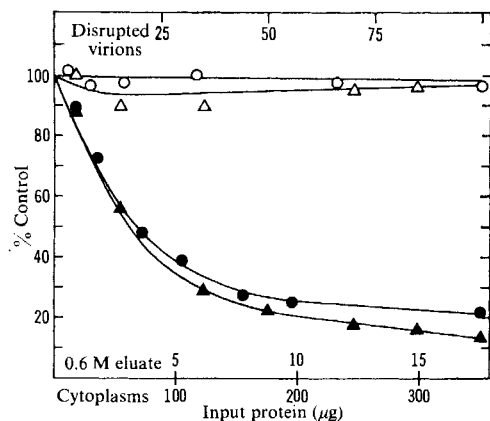


Fig. 3 Radioimmune-inhibition assay of Ad 2 T antigen activity. In the inhibition reaction, aliquots of 125 μg of an immunoglobulin mixture from anti-Ad 2 T ascitic fluid (50 μg) and from a normal hamster serum pool (75 μg) were preincubated with increasing amounts of unlabelled 0.6 M eluate (infected cells), unlabelled cytoplasm from infected or mock-infected cells, or disrupted Ad 2 virions, prepared as described in Fig. 1. Incubation was for 30 min at 37 °C in 400 μl of reaction mixture containing PBS-EDTA, 1% desoxycholate, and 1% Triton X-100. Control tubes consisting of the same mixture of immunoglobulins described above were preincubated with buffer alone. ³H-eluate (13,049 c.p.m., 10,000 c.p.m. μg⁻¹) was then added and the tubes were incubated for an additional 30 min. Goat anti-hamster IgG serum (50 μl) was added and incubation was continued for an additional 2 h at 37 °C. The precipitated complexes were then treated as described in Fig. 2 and counted. ○, Disrupted Ad 2 virions; ●, 0.6 M eluate (infected cells); △, cytoplasm from mock-infected cells; ▲, cytoplasm from infected cells. The different protein inputs are given on the abscissae. The degree of inhibition (% control) was calculated as follows after subtraction of background c.p.m. (nonspecific precipitation with normal hamster immunoglobulin) from both inhibition and control reactions:

$$\% \text{ control} = \frac{\% \text{ input c.p.m. precipitated in inhibition reaction}}{\% \text{ input c.p.m. precipitated in control reaction}} \times 100$$

- 8 Walter, G., and Maizel, J. V., Jr, *Virology*, **57**, 402-408 (1974).
- 9 Green, M., *Rev. Biochem.*, **39**, 701-756 (1970).
- 10 Freeman, A. E., et al., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1205-1212 (1970).
- 11 Graham, F. L., Van der Eb, A. J., and Heineker, H. L., *Nature*, **251**, 687-691 (1974); Graham, F. L., et al., *Cold Spring Harb. Symp. quant. Biol.*, **39** (in the press).
- 12 Gallimore, P. H., Sharp, P. A., and Sambrook, J., *J. molec. Biol.*, **89**, 49-72 (1974).
- 13 Litman, R. M., *J. biol. Chem.*, **248**, 6222-6233 (1968).
- 14 Maizel, J. V., Jr, in *Fundamental Techniques in Virology* (edit. by Habel, K., and Salzman, N. P.), 334-362 (Academic, New York and London, 1969).
- 15 Green, M., and Pina, M., *Proc. natn. Acad. Sci. U.S.A.*, **15**, 1251-1259 (1964).
- 16 Prage, L., Pettersson, V., and Philipson, L., *Virology*, **36**, 508-511 (1968).
- 17 Sever, J. L., *J. Immun.*, **88**, 320-329 (1962).
- 18 Horwitz, M. S., and Scharff, M. D., in *Fundamental Techniques in Virology* (edit. by Habel, K., and Salzman, N. P.), 297-315 (Academic, New York and London, 1969).
- 19 Fahey, J. L., and Terry, E. W., in *Handbook of Experimental Immunology* (edit. by Wein, D. M.), 19-43 (Davis, Philadelphia, 1967).
- 20 Büttner, W., Veres-Molnar, Z., and Green, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2951-2955.
- 21 Alberts, B. M., and Frey, L., *Nature*, **227**, 1313-1318 (1970).
- 22 Wall, R., Weber, J., Gage, Z., and Darnell, J. E., *J. Virol.*, **11**, 953-960, 1973.
- 23 Carroll, R. B., Hager, L., and Dulbecco, R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3754-3757 (1974).
- 24 van der Vliet, P. C., Levine, A. J., Ensinger, M. J., and Ginsburg, H. S., *J. virol.*, **15**, 348-354 (1975).

Paramagnetic species in cataractous human lenses

KURZEL *et al.*¹ studied the low temperature phosphorescence emission from normal and cataractous human lenses at 77 K. After comparing their lens data with those obtained from tryptophan in a frozen ethanol-water glass, they concluded that the phosphorescence of the lens is caused by the tryptophan residues of lens proteins. This was based on their finding in the lens a phosphorescence $\lambda(\text{max})$, phosphorescence excitation and phosphorescence quantum yield consistent with tryptophan and differing significantly from those of phenylalanine and tyrosine. Here we present the results of an electron spin resonance (ESR) study which characterises the paramagnetic triplet state of normal and cataractous human lenses and provides proof that it stems from the tryptophan moiety and not tyrosine or phenylalanine. This supplements previous observations that tryptophan is the dominant species in cataractous human lenses, giving rise to phosphorescent emission. In addition, we have discovered that the lens excited triplet (phosphorescent) tryptophan is associated with the production of a free radical; we believe that this free radical may be the missing link between ultraviolet irradiation and lens photodamage leading to cataract formation^{3,4}.

ESR measurements were made with a Varian E-4 spectrometer. The ultraviolet light source consisted of a Bausch and Lomb Model SP-200 mercury lamp which was focused by quartz optics on to the irradiation slits in the ESR cavity. The microwave power was set at 10 mW or 100 mW for all ESR measurements and the microwave frequency ranged between 9.089 and 9.093 GHz.

Cataractous lenses were obtained intact from surgery and noncataractous *post mortem* lenses were obtained intact at autopsy. The lenses were frozen immediately in liquid nitrogen and stored at 77 K. Before ESR measurements, the lenses were thawed, and each lens nucleus was removed and inserted into a quartz sample tube (3 mm inner diameter); the samples were then refrozen to 77 K.

Aromatic amino acid samples (0.1 mM) were prepared by dissolving them in solvents consisting of equal volumes of 0.1 M phosphate buffer (pH 7.0) and glycerol. The solutions were placed in quartz sample tubes (3 mm inner diameter) and frozen to 77 K. For ESR measurements, the samples were placed in a liquid nitrogen-filled finger Dewar which was subsequently inserted into the microwave cavity.

The triplet state ESR absorption derivative obtained in this study corresponds to the $\Delta M=2$ transition. The position of the peak, H_{min} , on the low field side of the spectrum is related to the zero-field-splitting parameter which characterises the spin-spin interaction between the two electrons which comprise the triplet. H_{min} and the linewidth of the ESR signal are both sensitive to the electronic structure of excited molecules and can be used to identify the triplet species.

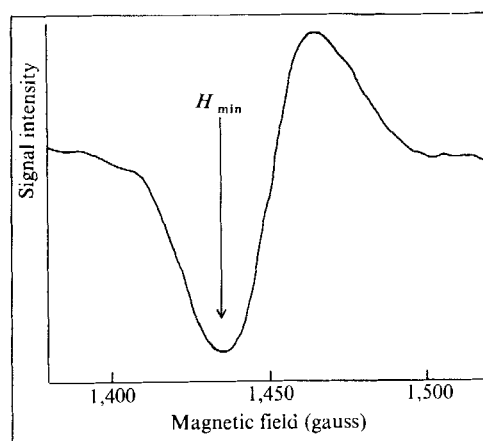


Fig. 1 Triplet state of ESR spectrum for the $\Delta M=2$ transition in the human lens. Temperature=77 K. Microwave frequency=9.092 GHz. Microwave power=10 mW.

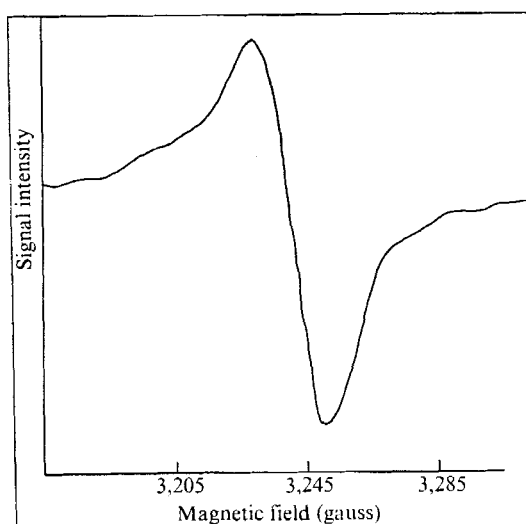
A typical triplet state ESR spectrum for the human lens is shown in Fig. 1. For a microwave frequency of 9.091 GHz, H_{min} was $1,435 \pm 5$ gauss and the peak-to-peak linewidth was 26 ± 2 gauss. No difference in either H_{min} or linewidth was observed between normal and cataractous lenses.

For the frozen tryptophan, tyrosine and phenylalanine solutions at 77 K, we obtained H_{min} and linewidth values of $1,430 \pm 5$ gauss, $1,240 \pm 5$ gauss, $1,225 \pm 5$ gauss and 25 ± 2 gauss, 30 ± 2 gauss, 60 ± 2 gauss, respectively. We take the close agreement between the lens and tryptophan parameters as conclusive evidence that the lens protein phosphorescent state originates from the tryptophan residues; Shiga and Piette⁵ reached a similar conclusion for ovalbumin and bovine serum albumin.

The triplet state lifetime of both normal and cataractous lenses was then measured; a difference would be interpreted to reflect an alteration in the microenvironment of tryptophan between normal and cataractous lenses⁶. The signal-to-noise ratio in the finger Dewar was not adequate to measure triplet state lifetimes, therefore, a Varian Model E257 variable temperature accessory was used. At 133 K, the phosphorescent triplet state in both normal and cataractous lenses was found to have a lifetime of 4.0 ± 0.1 s.

While making our ESR measurements, we observed that the intensity of the triplet state signal from both lenses and tryptophan solutions decreased after prolonged ultraviolet irradiation. Along with the decrease in signal intensity, a gradual brunescence coloration developed in the samples which had previously

Fig. 2 ESR spectrum of the ultraviolet induced free radical in the human lens. Temperature=77 K. Microwave frequency=9.092 GHz. Microwave power=100 mW. The g factor=2.005.



been uncoloured. We believe this coloration can be attributed to the presence of free radical products.

To test for the formation of free radicals, we irradiated a normal lens sample in the low temperature ESR finger dewar. We were able to follow the growth of the free radical species shown in Fig. 2. We have not been able to identify the radical species but assume that it results from the two photon ionisation of tryptophan. This has been demonstrated previously in frozen tryptophan solutions⁷.

Human lenses are known to become more coloured with age and contain an increased quantity of insoluble protein, leading to loss of transparency. Current theories suggest that this coloration and decreased transparency may be related to photo-induced changes in aromatic amino acids^{2,6,9}. Recently, photo-oxidation products of tryptophan have been identified in the human lens^{10,11}. Our data suggest that a mechanism for photoinduced lens damage might be the production of free radicals. Roubal and Tappel¹² have shown that free radicals induce protein polymerisation and loss of solubility. Free radical reactions have also been implicated in ageing¹³. There is reason to believe that ageing and cataractous lenses present the proper conditions for the stabilisation of free radicals. We have shown that ascorbic acid, which is a known free radical scavenger, is lost from the lens nucleus during cataract formation (unpublished data). The percentage of hydrophobic proteins also increases in the ageing lens¹⁴, which may permit trapping of free radicals¹⁵.

The possibility that ultraviolet light may be implicated in photodamage to the human lens is important, requiring further study. This is especially necessary in view of the widespread use of ultraviolet light today.

We thank the staff of the Ophthalmology Department at the National Naval Medical Center for assistance with the collection of lenses.

JOHN J. WEITER
EDWARD D. FINCH

Naval Medical Research Institute,
Bethesda, Maryland 20014

Received January 22; revised February 27, 1975.

- ¹ Kurzel, R. B., Wolbarsht, M. L., and Barkman, R. F., Yamanashi, B. S., Staton, G. W., *Nature*, **241**, 132-133 (1973).
- ² Kurzel, R. B., Wolbarsht, M. L., and Yamanashi, B. S., *Expl Eye Res.*, **17**, 65-71 (1973).
- ³ Zigman, S., Yulo, T., and Schultz, J., *Ophthalm. Res.*, **6**, 259-270 (1974).
- ⁴ Zigman, S., Schultz, J., and Yulo, T., *Expl Eye Res.*, **15**, 201-208 (1973).
- ⁵ Shiga, T., and Piette, L. H., *Photochem. Photobiol.*, **3**, 223-230 (1964).
- ⁶ Konev, S. V., *Fluorescence and phosphorescence of proteins and nucleic acids* (Plenum, New York, 1967).
- ⁷ Steen, H. B., *Photochem. Photobiol.*, **9**, 479-492 (1969).
- ⁸ Zigman, S., *Science*, **171**, 807-809 (1971).
- ⁹ Pirie, A., *Invest. Ophthalm.*, **7**, 634-650 (1968).
- ¹⁰ Pirie, A., *Israel J. med. Sci.*, **8**, 1567-1573 (1972).
- ¹¹ Van Heyningen, R., *Expl Eye Res.*, **15**, 121-126 (1973).
- ¹² Roubal, W. T., and Tappel, A. L., *Archs Biochem. Biophys.*, **113**, 150-155 (1966).
- ¹³ Harman, D., and Piette, L. H., *J. Geront.*, **21**, 560-565 (1966).
- ¹⁴ Spector, A. et al., *The Human lens—in relationship to cataract* (Associated Scientific Publishers, New York, 1973).
- ¹⁵ Roubal, W. T., *J. Am. Oil Chem. Soc.*, **47**, 141-144 (1970).

Electron diffraction studies of biological membranes and lipids

IN diffraction studies of biological membranes, X-ray diffraction has been used more frequently than electron diffraction in spite of certain advantages inherent in the latter technique. Electron diffraction enables the study of a small area (about $1 \mu\text{m}^2$) of a very thin specimen, and the collection of experimental data within a short time. Moreover, the morphology and degree of purity of the specimen can often be investigated. It seems possible to overcome the main problems arising from the application of the electron diffraction technique to biological materials, namely the difficulty of studying hydrated materials and the damage caused to the specimen by the electron beam, and a study of wet biological membranes has been reported¹. In this investigation dry specimens have been studied. Radiation damage was largely overcome by using

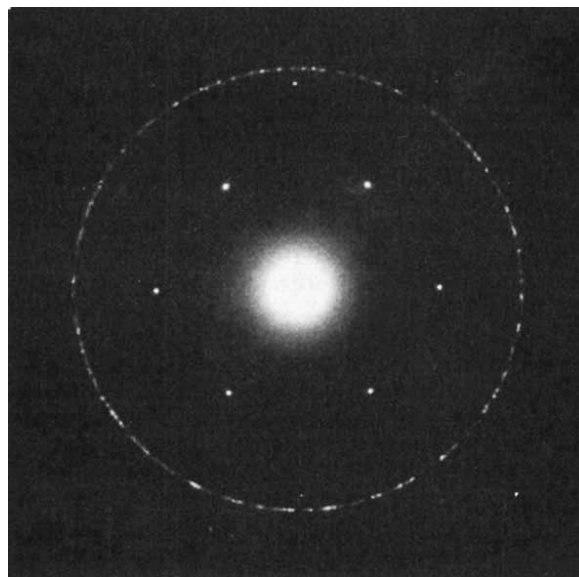


Fig. 1 Diffraction pattern of an unfixed and unstained *A. laidlawii* membrane. The two rings originate from the aluminium support film. The organisms were grown in a lipid-depleted basal medium supplemented with elaidic acid², and the membranes were isolated by osmotic lysis and washed³. After the last washing the membranes were resuspended in 10 mM ammonium acetate and deposited on copper grids covered with a 10 nm thick support film of aluminium. Membrane lipids were extracted with chloroform-methanol (2:1), freed from non-lipid contaminants⁴ and fractionated into neutral, phospho- and glycolipids on a silicic acid column (BioSil HA). Lipid specimens for diffraction studies were prepared by spreading a lipid solution on a water surface and depositing the film obtained on grids, which were either bare or covered with an aluminium film. Stearic acid specimens were prepared in the same way.

minimum beam current, by maximally overfocusing the first condenser of the electron microscope, and by moderately overfocusing the second condenser. To minimise the temperature rise of the specimen, caused by inelastically scattered electrons, a cooled specimen holder was used. To facilitate heat transport, the specimen grids were covered with aluminium instead of carbon. A Philips 301 G electron microscope equipped with an anti-contamination device was used and the diffraction patterns were recorded at 100 kV on a highly sensitive X-ray film, Kodirex. All diffractograms were recorded at a specimen holder temperature of about -60°C , which was regarded as a fair estimate of the temperature of the specimen itself.

Diffraction patterns of *Acholeplasma laidlawii* membranes (Fig. 1) showed the same pattern geometry as the stearic acid and the isolated lipids. The patterns were obtained only from small observation areas, about $1 \mu\text{m}^2$. Nevertheless, superimposed, disoriented patterns could occasionally be seen. When larger areas were chosen, the observed patterns transformed into typical powder diagrams. The disoriented patterns might have derived from superimposed membranes or from a few domains within the same membrane possessing different orientations. The powder diagrams obviously originated from several membranes present in the observation area.

The diffraction patterns showed three spacings: 2.49, 3.67 and 4.15 Å, with an interplanar angle of 90° between the 2.49 and 3.67 reflections, and an angle of 68° between the 4.15 Å reflections. These data suggest an orthorhombic unit cell having an *a* axis of 7.37 Å and a *b* axis of 4.98 Å. Larger and slightly less reproducible spacings, about 2.6, 4.1 and 4.6 Å, were observed in preparations washed with buffers containing calcium or magnesium ions. It therefore

seems reasonable to assume that the presence of divalent cations caused the larger spacings in less exhaustively washed membranes. Interactions between these ions and the phospholipids would thus be the most feasible ones.

To investigate the influence of dehydration on the structure of the lipids in the *Acholeplasma* membranes, an X-ray powder diffractogram was taken, using a Guinier camera. The same spacings as in the diffractograms described above were obtained. This indicates that the water content of the membrane is of minor importance for the lipid structure below the thermal transition temperature.

The distances and angles characterising the diffractogram presented here are rather close to the parameters of the proposed hexagonal structure of biological membranes above the transition temperature^{1,2,6}. The latter structure could be obtained by small changes in the 3.67 and 2.49 Å distances reported above. Thus our results show that diffraction studies of dry, single membranes in the static state prevailing below the thermal transition temperature are of importance for our understanding of the dynamic state characterising the membranes above this temperature. The finding that the 3.67 Å reflection is probably caused by lipids and not by proteins as proposed earlier² should also be mentioned in this connection.

We thank Dr S. Andersson for the use of the Philips EM 301 G microscope and for discussions, and Professor C. Weibull for reading the manuscript.

E. CARLEMALM
Å. WIESLANDER

Department of Microbiology,
University of Lund,
S-223 50 Lund, Sweden

Received February 21, 1975.

- ¹ Hui, S. W., and Parsons, D. F., *Science*, **184**, 77-78 (1974).
- ² Razin, S., and Rottem, S., *J. gen. Microbiol.*, **33**, 459-470 (1963).
- ³ Engelman, D. M., *J. molec. Biol.*, **58**, 153-165 (1971).
- ⁴ Wells, M. A., and Dittmer, J. C., *Biochemistry*, **6**, 1259-1263 (1963).
- ⁵ Bernengo, J. C., and Simpkins, H., *Can. J. Biochem.*, **50**, 1260-1266 (1972).
- ⁶ Luzzati, V., *Biological Membranes* (edit. by Chapman, D.), 71-123 (Academic, New York, 1968).

Primordial origins of chirality

THE origin of optical asymmetry in relation to the origin of life has been extensively discussed¹⁻⁴. One suggestion is that there is a connection between molecular asymmetry and the asymmetry of elementary particles produced by weak interactions. Various mechanisms have been proposed whereby the choice of one optical isomer could be influenced by longitudinally polarised (left handed) electrons from β decay^{5,6}, but early experiments did not give any significant results⁷. Later, both positive^{8,9} and negative¹⁰⁻¹² results have been obtained in a variety of experiments (differential decomposition of enantiomers bombarded by β^- -rays, spin-polarised positron and muon annihilation in enantiomers).

In a new approach to this problem we carried out the crystallisation of DL-NaNH₄ tartrate in the presence and absence of chiral β^- particles from ³²P. Below 27 °C, DL-NaNH₄ tartrate crystallises in racemic conglomerates¹³, that is, D and L crystallisation centres form independently and then grow. Any difference between the number of D and L centres will have a more marked effect on the optical activity of the conglomerates as crystallisation proceeds.

Pure DL-tartaric acid, free of mesotartaric acid, was converted to the sodium ammonium salt. Its optical activity was checked by measuring α_{280} with a JASCO ORD/UV-5 spectropolarimeter, and θ_{225} with a JASCO 40c dichrograph. The sensitivity of the latter is ± 0.1 m°, and a 1×10^{-6} M excess of one isomer could have been detected. In the starting racemic material 2×10^{-6} M predominance of D (+) isomer was found.

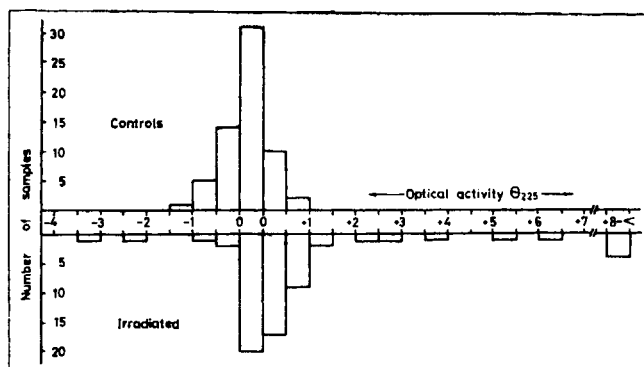


Fig. 1 Distribution of levels of optical activity in NaNH₄-tartrate samples as crystallised in the absence (upper part of diagram) and in the presence (lower part) of chiral β^- particles (63 crystallisations in both cases). Positive CD signal at 225 nm (θ_{225}) corresponds to a negative rotation at the sodium D line, which is characteristic of the unnatural L (—) isomer.

For crystallisation, 8 ml 45% solutions of the tartrate were used. In six series (in all, 63 independent crystallisations) K₃³²PO₄ was added, the level of radioactivity being 20 μ Ci ml⁻¹; in the six control series, inactive K₃H³¹PO₄ was added. The pH in all series was adjusted to 9.5 with NH₄OH. After equilibration at room temperature crystallisation was carried out over P₂O₅ in a desiccator at 1-4 °C. Precautions were taken in cleaning the glassware, excluding dust, and so on. When the crystallisation was about half complete (8-12 d), the crystals were filtered and weighted, and their optical activity measured in 2×10^{-2} M aqueous solution. As a check, the optical activity of the supernatant was also measured.

Figure 1 shows that in the presence of chiral electrons (³²P) the unnatural L (—) salt crystallised preferentially whereas an approximately symmetrical distribution is obtained in the control series, in the series with ³²P there is a markedly asymmetric distribution. It is clear, however, that β^- particles shifted the crystallisation towards L (—) isomer. The shift is significant at the 0.1% level (both χ^2 and Student's *t* test). The magnitude of the shift is indicated by the medians: 0.0 for control series, and 0.2 for irradiated samples. As would be expected, the small predominance of the D (+) isomer in starting material very slightly shifted the crystallisation of the control samples towards D (+) isomer.

The exact mechanism of the effect is not known. We think it unlikely that β rays would influence crystal growth, and suggest that the influence was to enhance the number of crystallisation centres of the L isomer. In this connection it should be mentioned that hydrated electrons have quite a long lifetime, particularly in alkaline solutions¹⁴.

We thank Dr Cs. Fajsz, Dr. Soós and L. Kovács for their help.

K. L. KOVACS
A. S. GARAY

Biological Research Center,
6701 Szeged, POB 521, Hungary

Received January 23, 1975.

- ¹ Ulbricht, T. L. V., in *Comparative Biochemistry*, 4 (edit. by Florkin, M., and Mason, H. S.), (Academic, New York, 1962).
- ² Bonner, W., in *Exobiology* (edit. by Ponnampetuma, C.) (North-Holland, New York, 1972).
- ³ Thiemann, W., and Darge, W., *Origin of Life*, 5, 263 (1974).
- ⁴ Ulbricht, T. L. V., *Origin of Life* (in the press).
- ⁵ Vester, F., Ulbricht, T. L. V., and Krauch, H., *Naturwissenschaften*, **46**, 68 (1959).
- ⁶ Ulbricht, T. L. V., *Q. Rev. Biol.*, **13**, 48 (1959).
- ⁷ Ulbricht, T. L. V., and Vester, F., *Tetrahedron*, **18**, 629 (1962).
- ⁸ Garay, A. S., *Nature*, **219**, 338 (1968).
- ⁹ Garay, A. S., Keszthelyi, L., Demeter, I., and Hrasco, P., *Nature*, **250**, 332 (1974).
- ¹⁰ Goldanskii, V. I., and Khrapov, V. V., *Soviet Phys. JETP*, **16**, 582 (1963).
- ¹¹ Bernstein, W., Lemmon, R., and Calvin, M., in *Molecular Evolution* (edit. by Rohlfing, L., and Oparin, A. I.), (Plenum, New York, 1972).
- ¹² Lemmon, R. M., et al., *Nature*, **252**, 692 (1974).
- ¹³ Eliel, E. L., *Stereochemistry of Carbon Compounds*, 44-45 (McGraw-Hill, New York, 1962).
- ¹⁴ Streffer, C., *Sirahlen Biochemie* (Springer, Berlin, 1969).

reviews

To review one volume of Needham's great monument-in-the-making is like trying to make an architectural assessment of a single pillar of a temple. But one is encouraged in the attempt because a pillar is not a bad analogy. The superstructure of modern science is sustained on many singular supports; Greek atomism, mediaeval astronomy, Renaissance navigation, and so on. One of the pillars, alchemy, changes its appearance as one changes one's viewpoint.

There has been a lot of change in the historical assessment of alchemy. For a time it was viewed as the main forerunner of modern scientific chemistry; then as mere superstition and charlatanism; then as a lesser light to technological chemistry. Now we are beginning to re-assess it yet again as something respectable in its own right, a body of practice and belief that should be viewed not in terms of modern science and modern society, but as something of its own time. It is one of the outstanding merits of Needham's work that at the same time as he makes us look for the first time at a culture we did not pretend to know, he also makes us look afresh at the western science we thought we did know. This is true in general of his whole enterprise, but particularly clear in this volume.

It is at once chastening and stimulating to see Chinese alchemy studied with an eye to chemical detail which has not been applied so closely by any writer about western alchemy in the present generation of historians of western science.

Alchemy was concerned with many things, but the big thing was the distinctive character of gold. Gold was astonishing stuff to the ancient world. Familiarity with it never bred contempt, because one characteristic marked it off from all else; it was incorruptible. It survived all trials and could be recovered pure and undiminished from alloys and disguises by the process of cupellation. True: it was beautiful, but other things were beautiful. The imitation of its beauty was one thing: aurification, the production of the appearance of gold. But could gold be made: was true aurification a possibility? And if one form of matter could be incorruptible, could not Man be made incorruptible? So far, so simple. These are the familiar themes of western alchemy. The value

Science ancient and modern

Frank Greenaway

Science and Civilisation in China. Vol. 5: Chemistry and Chemical Technology. Part 2: Spagyric Discovery and Invention: Magisteries of Gold and Immortality. By Joseph Needham with Lu Gwei-Djen. Pp. xxxii+510. (Cambridge University Press: London, October 1974.) £12.00; \$35.00.

of Needham's work lies in his expounding Chinese alchemy as a thing distinct in itself, having its own moral values, social relations and methods of enquiry. If some resemblances, some identities, are discovered which show Chinese and western alchemy to have a common basis and to have arrived at some common conclusions, this is for a good reason: "While we can easily see that artistic styles and expressions, religious ceremonies and doctrines, or different kinds of music, have tended to be incommensurable; for mathematics, science and technology the case is altered—man has always lived in an environment essentially constant in its properties, and his knowledge of it, if true, must therefore tend towards a constant structure".

Is the limited career of Chinese alchemy to be considered in Spenglerian terms, an aspect of a quasi-organic cultural growth and decay, or in Kuhnian terms, an aspect of the adoption of a compelling fashion of thought in a line of succession without progress? Needham respectfully rejects the Spenglerian pessimism but will not accept a naive Kuhnism. In fact, he will not accept entire any of the currently packaged philosophies of history, but insists that he is engaged in an enquiry which will lead to a new appreciation of the origins, development and internal and external relations of Chinese science when the work is done. His study of Chinese science leads him to remind us how provisional is our modern scientific 'knowledge'.

"It is neither independent of the accidents of Western European history, nor is it a final court of appeal for the eschatological judgment of the value of past scientific discoveries

either in West or East. It is a reliable measuring stick so long as we never forget its transitory nature."

One of the most difficult exercises for the historian of science is to relate the techniques of a historical period to modern scientific and technical knowledge. Needham's treatment of the metallurgical practices which the alchemists used for precious and base metals is exemplary. These pages can be recommended to any reader, whether he cares about Chinese alchemy or not, who wants a brief, logical (and so far as I can gauge, pretty complete) survey of all early metallurgical developments. There are some surprises, such as the widespread use of cupronickel, the as yet unexplained occurrence of some specimens of aluminium, and the extent of the trade in zinc.

When Needham has to deal with the derivative human theme of alchemy, the elixir of life, the drug of deathlessness, he does so by relating it most skilfully to the technical, metallurgical theme. He shows how the idea of physical immortality emerged almost imperceptibly out of the idea of longevity. There is, however, no one persistent view to be shown. There were many religions in China, influential successively or simultaneously, and each had its own philosophy of the nature of human life and its prolongation. In each, alchemical ideas could play a part. The descriptions of religious observance, incense burning in particular, are very well done, the accounts of unfamiliar liturgical and ritual customs never being obscured by the inevitably complex detail.

The excellence of the scholarly apparatus of these successive volumes is well known and needs no further praise. Nor does Needham's unfailing courtesy and generosity to his collaborators, whether they contributed to a whole chapter or a single line.

The value of this volume, only one of the four projected for the whole of Chinese chemistry, is likely to be as much in its stimulus to the reappraisal of western alchemy as in its original *raison-d'être*. A successful and convincing history of scientific chemistry as a whole has yet to be written. It would be strange, yet somehow satisfactory, if the stimulus to a new approach to a critique of modern chemical science should come from this splendid study of things "far away and long ago".

Biology of the gene

Gene Expression. Vol. 1: *Bacterial Genomes*. Pp. xvii+642. June 1974. £4.75. Vol. 2: *Eucaryotic Chromosomes*. Pp. xiv+467. September 1974. Benjamin Lewin. £8.00, cloth; £3.95, paper. (Wiley, London and New York.)

GENE EXPRESSION, originally intended as a revision of Benjamin Lewin's earlier successful treatise, *The Molecular Basis of Gene Expression*, reflects the growth of knowledge in the field and has expanded to become two major volumes.

Volume 1, *Bacterial Genomes*, reviews the extensive progress in the molecular genetics of prokaryotes over the past five years. The coverage is not limited strictly to gene expression, but includes the closely allied topics of recombination, DNA replication and cell division. The biochemically biased exposition, intended for advanced students and specialists, demands familiarity with both microbial genetics and nucleic acid biochemistry.

Lewin presents his subject through a critical appraisal of the key experiments, occasionally including carefully selected data. The clarity of the presentation is enhanced by the well designed diagrams. Original papers are abundantly cited; curiously though, references to the decisive experiments seem too often absent.

Volume 2, *Eucaryotic chromosomes*, contrasts strikingly with Volume 1 in the lack of precise information deriving, quite naturally, from the relative complexity of the subject. Lewin argues that sufficient advances have been made in our knowledge of eucaryotic gene expression to justify this attempt at proposing "a preliminary conceptual framework".

The first part of Volume 2 is devoted to the ultrastructure of eucaryotic chromosomes and its variation during the life cycle. The very lengthy discourse palls at times, but is alleviated by many excellent electronmicrographs. The analysis of DNA structure from the kinetics of renaturation and hybridisation is well explained, but the need for a sharper tool with which to approach sequence organisation in eucaryotic DNA is apparent between the lines.

The second part of the book is more directly concerned with the expression of eucaryotic genes. The processing of primary transcripts and models for the control of eucaryotic gene expression are prominent. The volume ends with an interesting discussion of the interaction between nucleus and cytoplasm, as studied by nuclear transplantation and somatic cell hybridisation.

Despite Lewin's apologies for his

selectivity, in both volumes he could reasonably have been more selective and thereby more concise. Certain experiments described in great detail are superseded by subsequent reports. Even elegant experiments which have been conclusively refuted are best forgotten. In each volume, after such an array of experimental observations and conclusions, I would have welcomed an attempt to present a wider view, putting recent developments into perspective and discussing their implications for the future. I felt it a pity, too, that the concentration on mammalian cells in Volume 2 meant the omission of those eucaryotic systems most amenable to genetic analysis and, therefore, most likely to yield important information on gene structure and expression.

These minor criticisms should not be taken to detract seriously from the obvious merit of these volumes, the depth, scope and modernity of which will ensure their value to everyone interested in the biology of the gene.

W. J. Brammar

Watching volcanoes

Physical Volcanology. (Developments in Solid Earth Geophysics, vol. 6.) Edited by L. Civetta, P. Gasparini, G. Luongo and A. Rapolla. Pp. xvi+333. (Elsevier Scientific: Amsterdam, Oxford and New York, 1974.) Dfl.90; \$34.75.

THIS book represents the combined efforts of four editors and seventeen contributors to mark the occasion of the retirement in 1971 of Professor Giuseppe Imbo from the directorship of the Vesuvian Observatory.

Like all commemorative books it is very conservative; thus we see again the classic investigations of volcanic seismicity from Japan and of crustal deformation from Hawaii.

That wouldn't matter if the UNESCO book on the same subject had not been published in 1971, but this new book repeats (verbatim in some instances) some 30% of that material.

And the title seems to me to be wrong. The book deals mainly with the application of instrumental techniques in all but two of the fourteen chapters, and though that makes for an excellent reference book, it rather avoids consideration of the underlying physics.

I think that a more interesting volume could have been written under the title of 'Physical Volcanology' if some attention had been given to the physical processes of volcanic phenomena. The physics of lava flows, cone formation, and magma degassing are hardly mentioned, and the more avant garde subjects like the influences of earth tides and local tectonics on eruptions are not seen.

The book, however, contains some very useful reference material for anyone actively engaged in volcanic research. Most of the chapters describe in detail the application and limitations of one or other of the geophysical and chemical techniques that have been successfully used on volcanoes, in each case by one of the contributors, all of whom are acknowledged experts. It is to be hoped, though, that they are not led by the title of Chapter 9 into confusing ashes for gases; and I hope too that their efforts may help avert the great ions (p. 317) resulting from large lava flows.

James L. Brander



Centenarian from a village near Sukhumi on the Black Sea coast. From *Biological Anthropology (Readings from Scientific American)*. With Introductions by Solomon H. Katz. Pp. 494+340 illustrations. (Freeman: San Francisco, 1975.) \$14.95, cloth; \$6.95, paper.

More than most will remember

Collected Papers of Sir Harold Jeffreys on Geophysics and Other Sciences. Vol. 2: *Observational Seismology*; Pp. xxi + 697; £20.10. Vol. 3: *Gravity*; Pp. xviii + 661; £19.60. (Gordon and Breach, London, Paris and New York, 1975.)

HAROLD JEFFREYS is truly one of the giants of 20th century science, although he ploughed a fairly lonely furrow between the wars at a time when bright young men were expected to go into atomic and nuclear physics. But now geophysics is the most fashionable of subjects and many of its practitioners rely, consciously or unconsciously, on the foundations that Jeffreys built. His writings both on geophysics and beyond (even to an appraisal of the psychoanalytic significance of a label on a beer bottle) have been both prolific and a delight to read. The seven textbooks are easily accessible, but the rest is scattered around more than 40 different journals. So is there maybe a case for the publication of his collected papers?

For all that Jeffreys has published widely, four fifths of the papers in these volumes (of the six that will ultimately appear) come from the *Monthly Notices of the Royal Astronomical Society* and its *Geophysical Supplement* (later the *Geophysical Journal*). (There were times when Jeffreys practically kept the *Geophysical Supplement* going single-handedly!) Thus, if you have access to a complete run of these two journals you are paying a lot for the other one-fifth of his published papers. And the editing is relatively slight.

One of the fascinations of science is the way things age rapidly—particularly the interpretation of observations; it is, then, of interest to learn what, in retrospect, scientists would stand by, and what they think is no longer tenable. For instance, I should have liked to know how Jeffreys thought the J-B tables of 1940 stood now that there are newer tables by Herrin (at least, I do know, but I wish he'd put it in print). But where Jeffreys does add a modern footnote—and this is infrequently—it is brief and not very helpful. For example: "Some stations, mainly southern ones, received much higher reliabilities when the ellipticity of the Earth was allowed for", at the end of a 20 page paper.

One footnote, though, is pure Harold Jeffreys and touches on the question of the WKB approximation. In a 1969 note on a section in a 1915 paper, Jeffreys writes "This was my first discovery of what is best described as the Green-Liouville approximation. I

rediscovered it in 1923, having twice forgotten Green's equivalent treatment of tidal waves in a canal of slowly varying sections". He has forgotten more than most of us will remember.

Alas, the price of the complete set is such that the only geophysicists with that sort of money to spare are not those who will think of acquiring these volumes. And librarians can with justification say that all the papers are accessible somewhere—except perhaps the beer-bottle one.

David Davies

Marihuana

Marijuana: Effects on Human Behaviour. Edited by Loren L. Miller. Pp. xiv + 405. (Academic: New York and London, December 1974.) \$29.50; £14.95.

The Use of Marijuana: A Psychological and Physiological Inquiry. Edited by Jack H. Mendelson, Michael Rossi and Roger E. Meyer. Pp. x + 202. (Plenum: New York and London, 1974.) £17.00; \$44.00.

OVER the last few years, research on cannabis has burgeoned astonishingly. This has not come about by the slow process of natural evolution, but very obviously because grant money has flowed in that direction, particularly in the United States and Canada. For anyone with a really well developed eye for a bandwagon, the ultimate would now be to obtain grant money for a study of the social and political determinants of this granting. The story would provide a fascinating case-study.

When, for basically political reasons, a great deal of research money is suddenly pushed in a particular direction the prognosis is always uncertain—the result may be a lot of mediocre research or, alternatively, rather good things may happen. With research into the effects of cannabis the outcome has been of the latter and more fortunate kind. In several different scientific areas cannabis research is beginning to have about it a modest feeling of excitement.

The range of this research and something of the promise, is well conveyed in the volume on cannabis which is edited by Dr Loren Miller. Edited volumes on cannabis are now appearing thick and fast, but so far this is undoubtedly the most interesting and authoritative array of papers on this topic to have been put together in one book. The 14 chapters each either report the contributor's original research with adequate delineation of context, or review a particular sector. The laboratory dissection of the psychological aspects of cannabis is the theme

of several different chapters, and one feature of recent work is that experiment on the drug's interference with complex mental functions is providing a useful tool for basic study of those types of function. Chapters on the influence of cannabis on memory and on attention, nicely illustrate this development. Other contributions at the laboratory end of the spectrum deal with neurophysiological research and possible interference with neurotransmitter mechanisms; again these are not just 'drug studies' but use of a particular substance as an investigating tool. We have moved on from the days when study of CNS function was largely based on physical ablation of its parts, but the analogy is close.

This book also deals with some more social-medical questions. A chapter on the drug and psychiatric illness, gives a particularly sensible appraisal of issues which have not always attracted the coolest discussion. Progression to other drugs, cannabis and violence, and cannabis and car driving, provide the matter for further chapters. Too easy an acceptance of the inevitability of that approach to research which is always one removed from the social reality (drinking experiments, as it were, in the simulated laboratory bar rather than at the Pig and Whistle), receives a healthy jolt from an account of driving research in which experimental subjects drove round town in dual control cars after smoking, with careful real-life observation being backed by physiological measures made at the same time.

In *The Use of Marijuana* Professor Mendelson and his colleagues give an account of a project which yields a mass of data on a number of separate aspects of the drug's effect on the human subject. Even leaving aside the specialism of the content, this is a book which deserves notice as a superbly competent piece of scientific investigation. Some years ago Mendelson and his collaborators made an important contribution to alcoholism studies by observation of, and multiple measures on, alcoholics drinking in an experimental ward, which provided an operant-conditioning paradigm: the same type of design has now been applied to the influence of cannabis on man. In tolerant individuals, marihuana intoxication sustained over a period of 3 weeks and with dose on occasion going up to 200 mg THC a day, produced remarkably little disturbance. As the investigators themselves say, their work leads to no closure of the cannabis debate but only points to the necessity for "more sophisticated questions". Any assertion that cannabis is a drug which cannot produce tolerance, is after this study certainly no longer credible. Griffith Edwards

Prosimians and evolution

Prosimian Biology. (Proceedings of a Meeting of the Research Seminar in Archaeology and Related Subjects.) Edited by R. D. Martin, G. A. Doyle and A. C. Walker. Pp. xxi+983. (Duckworth: London, November 1974.) £30.00.

"THIS collection of papers" the late W. C. Osman Hill wrote in the foreword, "can be seen to cover an extremely broad spectrum". He was far from wrong. Fifty four chapters by authors from many countries and several disciplines describe the behaviour, ecology, physiology, anatomy, neurophysiology, cytology, biochemistry and evolution of prosimians.

The book represents more than an addition to current knowledge of another group of little known mammals. As Doyle and Martin point out in their introductory chapter, the morphology of contemporary prosimians is broadly similar to that of the array of Eocene primates from which the Anthroidea developed. Living species may thus provide an approximate blueprint of the behaviour and ecology of the common ancestors of prosimians and anthropoids. Moreover, the many examples of convergence between prosimian and anthropoid species occupying similar niches strengthen arguments relating behavioural and morphological differences to variation in ecology. For both reasons, the study of prosimians may help us to understand the evolution of the higher primates.

The book's most important contribution lies in the first section. This reports the results of field studies covering 15 different species. Until recently, the behaviour and ecology of prosimians was poorly documented, partly because most species are nocturnal and almost all live in habitats where observation is difficult. This group of papers represents a major advance in the field and bears witness as much of the authors' endurance as to their perspicacity. The extensive sections on anatomy, biochemistry and evolution provide many insights into the functional significance of particular structures, ranging from teeth to toenails, and into the phylogenetic status of a wide variety of species. A general weakness, considering the stated justification for studying prosimians, is the authors' reluctance to discuss the relevance of their findings to the evolution of the higher primates and man.

The book is well produced and carefully edited. It contains fewer make-weight chapters than most published collections of conference papers. And



Lesser bushbabies (*Galago senegalensis moholi*)

it is likely to represent a milestone in its field for many years. Nevertheless, it should make contributors, editors and publishers think carefully about the wisdom of producing similar volumes. At £30.00 it is beyond the reach of private buyers. All except the most affluent libraries are likely to look askance at highly specialised symposium proceedings which cost as much

as a year's subscription to the average journal. If the size and cost of symposia proceedings continue to escalate at this rate, it is inevitable that they will only be available in the largest libraries. That will benefit neither readers nor authors.

T. H. Clutton-Brock

Most theories according to others

Transport Phenomena in Aqueous Solutions. By Tibor Erdey-Gruz. Pp. 512. (Adam Hilger: London; Akademiai Kiado: Budapest, December 1974.) £12.00.

THE transport properties covered in this book are the traditional ones of shear viscosity, concentration diffusion and electrical conductivity. There is only passing mention of bulk viscosity, thermal diffusion, dielectric relaxation, and so on. The three properties covered form the subjects of chapters 2, 3 and 4. The first chapter discusses the molecular structure of liquid water and the fifth, which should perhaps more logically have been the second, covers the equilibrium properties of aqueous solutions.

The author has an encyclopaedic knowledge of what has been said and done, but shows little discrimination. Every experiment and, what is worse, every theory is referred to, but he rarely tries to judge between them. Thus, sections 1.3.3.2 to 1.3.3.9 describe briefly eight different classes of theories of the structure of water and are followed, as if in despair, by 1.3.3.10 which is called simply "Further Theories". His classification is impeccable but he does not tell the reader which of these mutually inconsistent theories he

believes to be correct; nor does he marshal well the evidence on which the reader could judge for himself. The same is true of the treatment in later chapters on theories of transport. Moreover, even his references become perfunctory when he touches on modern theories which require for their understanding a knowledge of non-equilibrium statistical mechanics.

Perhaps he sees it as the duty of the author of a monograph simply to summarise the views of others (the commonest phrase in the book is "according to X"), and not to inflict his own views on his readers, but it makes for a dull book. The only point at which it comes alive is in the section (p. 351-390) on the effect of non-electrolytes on electrolytic conductivity. There, the author has himself contributed experimentally to the subject and his discussion is much more cogent than elsewhere.

The book can be recommended, however, to those who want access to the voluminous experimental literature, and to the mechanically more simple theories of the last 40 years. The references and indexes are, on the whole, accurate, comprehensive and organised in a way which makes it easy to recover the information in the literature.

J. S. Rowlinson

announcements

Awards

Her Majesty the Queen has approved the grant of a **Royal Charter** to the **Institute of Measurement and Control**.

The Royal Meteorological Society has announced the following awards: **Symons Memorial Gold Medal** to **B. J. Mason, FRS** (development of meteorology and understanding of the physics of clouds); **Fitzroy Prize** to **R. W. Gloyne** (application of meteorology and climatology to agriculture, horticulture and forecasting); **L. F. Richardson Prize** to **J. J. Barnett** (stratosphere on Nimbus IV).

The Royal Society has announced the election of the following Fellows: **R. J. H. Beverton, CBE; G. M. Binnie; E. G. Bowen, CBE; S. H. U. Bowie; G. M. Brown; A. D. Buckingham; P. C. Caldwell; J. Charnley, CBE; J. W. Christian; B. A. Cross; K. Dalziel; P. De Mayo; J. M. Dodd; A. Edrélyi; D. A. Haydon; G. N. Hounsfield; A. M. Lane; A. R. Lang; A. L. McLaren; R. Mason; C. Milstein; P. A. P. Moran; R. C. Rainey; E. C. Slater; R. A. Slatyer; D. C. Smith; B. P. Stoicheff; G. P. L. Walker; R. Weck, CBE; F. R. Whatley; R. Wilson; E. C. Zeeman.**

Appointments

Peter Palmer, a Director of Drake and Cubitt Holdings, has been appointed the first visiting professor in the department of mechanical engineering of the City University.

James Briden has been appointed professor of geophysics in the department of earth sciences at Leeds University.

International meetings

May 6–7, **Isolation and clinical significance of trace components of plasma**, Washington, DC (Symposium Planning Committee, American National Red Cross, Blood Research Laboratory, 9312 Old Georgetown Road, Bethesda, Maryland 20014).

May 13–14, **Pulsed methods in photochemistry**, London (Dr M. A. West, The Royal Institution, 21 Albemarle Street, London W1X 4BS, UK).

June 9–August 29, **Frontiers of science**—Gordon Research Conferences on a wide variety of physical and biological

topics, running throughout the summer, New Hampshire and California. Applications to: Dr A. M. Cruikshank, Director, Gordon Research Conferences, University of Rhode Island, Pastore Chemical Laboratory, Kingston, Rhode Island 02881; Programme Summary from: Women's Colby College, New Hampshire, New London, New Hampshire 03257.

June 16–18, **Mass spectrometry in biochemistry and medicine**, Alghero, Sardinia (Alberto Frigiero, c/o Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157 Milan, Italy).

June 16–20, **American Geophysical Union**, Washington, DC (Shelton Alexander, 204 Mineral Sciences Building, Pennsylvania State University, Pennsylvania 16802).

June 17, **Polymer science: achievements and prospects**, Pittsburgh (Hershel Markovitz, Department of Chemistry, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213). The symposium is in honour of Paul J. Flory, the Nobel Laureate in chemistry, 1974.

June 22–28, **Immunochemistry of the cell membrane**, Ravello, Italy (Dr P. Comoglio, Department of Anatomy, Turin University, Corso M. D'Azeglio 52, 10126 Torino, Italy, or Dr S. Zappacosta, Fondazione Pascale Cancer Institute, Via M. Semmola, 80131 Napoli, Italy).

June 23–July 4, **Physics of quantum electronics**, Santa Fe, New Mexico (Professor S. F. Jacobs, Optical Sciences Center, University of Arizona, Tucson, Arizona 85721).

June 25–27, **Mercury**, Pasadena, California (James A. Dunne, Executive Secretary, First International Colloquium on Mercury, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove, Pasadena, California 91103).

June 30–July 1, **Applications of enzymes**, Exeter (Miss M. V. Auguste, The Chemical Society, Burlington House, London W1V 0BN, UK).

June 30–July 4, **Marine natural products**, Santa Barbara, California (Paul J. Scheuer, University of Hawaii at Manoa, Department of Chemistry, 2545 The Mail, Honolulu, Hawaii 96822).

Reports and publications

Great Britain

Memoirs of the Royal Astronomical Society, Vol. 78, Part 2: Equivalent Width and Rotational Velocities of Southern Early-Type Stars. By L. A. Balona. Pp. 51–72. (Oxford and London: Blackwell Scientific Publications, 1975. Published for the Royal Astronomical Society.) [281]

The Stable Isotope Project—Progress Report 1970–1973. (Publications Series C, No. 13, October 1974.) Pp. iv + 16. (London: Natural Environment Research Council, 1974.) [291]

Bulletin of the British Museum (Natural History). Geology, Vol. 25, No. 4: Cretaceous Faunas from Zululand and Natal, South Africa, Introduction, Stratigraphy. By W. J. Kennedy and H. C. Klinger. Pp. 273–315 + 1 plate. (London: British Museum (Natural History), 1975.) £3.75. [291]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 277, No. 1272: Coupling Coefficients and Tensor Operators for Chains of Groups. By P. H. Butler. Pp. 545–585. UK £1.50. Overseas £1.60. Vol. 277, No. 1273: On the Use of the Breit-Pauli Approximation in the Study of Relativistic Effects in Electron-Atom Scattering. By M. Jones. Pp. 587–622. UK £1.30; Overseas £1.50. (London: The Royal Society, 1975.) [301]

Proceedings of the Royal Irish Academy. Vol. 74, Section A, Nos. 18–36: Spectral Theory Symposium. Pp. 133–310. £3.45. Vol. 74, Section B, No. 28: Some *Penniretepra* (Bryozoa) from the Visean on County Fermanagh with a Revision of the Generic Name. By Foluso Olaloye. Pp. 471–506 + plates 15–22. £1.53. No. 29: Enzyme Production by *Bacillus polymyxa* in an Extract of Peat. I. Preliminary Studies and the Effect of Added Carbon on Amylase and Protease Production. By W. M. Fogarty and P. J. Griffin. Pp. 507–522. 31p. Nos. 30 and 31: Enzyme Production by *Bacillus polymyxa* in an Extract of Peat. II. The Effects on Nitrogen and other Factors on Amylase and Protease Production. III. Amylase and Protease Production in Batch Cultures. By P. J. Griffin and W. M. Fogarty. Pp. 523–548. 46p. (Dublin: Royal Irish Academy, 1974.) [311]

Department of Industry: Laboratory of the Government Chemist. Report of the Government Chemist, 1973. Pp. iv + 175 + 8 plates. (London: HMSO, 1974.) £1.80 net. [311]

Medical Research Council. The Toxicity of Plutonium. Pp. iii + 38. (London: HMSO, 1975.) 45p net. [311]

Government Statistics: a Brief Guide to Sources. Pp. 18. (London: Central Statistical Office, Great George Street, SW1, 1975.) [32]

Mineral Resources Consultative Committee. Mineral Dossier No. 9: Tin. Compiled by D. Slater. Pp. vi + 41. 65p net. Mineral Dossier No. 10: Talc. Compiled by D. E. Higley. Pp. 25. 55p net. (London: HMSO, 1974.) [32]

University of Cambridge. Annual Report of the Institute of Astronomy, 1973/1974. Pp. 20. (Cambridge: The Observatories, The University, 1975.) [42]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 270, No. 901: A Physiological Analysis of Walking in the American Lobster (*Homarus americanus*). By D. L. Macmillan. Pp. 1–59. (London: The Royal Society, 1975.) UK £2.50; Overseas £2.60. [62]

Bird Life in the Royal Parks, 1973. (A Report by the Committee on Bird Sanctuaries in the Royal Parks.) Pp. 13. (London: Royal Parks Division, Department of the Environment, 29 Great Peter Street, SW1, 1975.) gratis. [62]

Materials for Cryogenic Service: Engineering Properties of Austenitic Stainless Steels. Pp. 48. (London: International Nickel, Ltd., 1974.) [62]

Estuaries Research: Report of the NERC Working Party on Estuaries Research. (Publications, Series B, No. 9.) Pp. 62. (London: Natural Environment Research Council, 27/33 Charing Cross Road, WC2, 1975.) [62]

Royal Observatory Bulletins No. 178: Provisional Positions of the Sun and Planets 1957–1971. By M. E. Buontempo, J. V. Carey and Patricia Eldridge. Pp. 191–300. (Hertsmoonceux: Royal Greenwich Observatory, 1973.) £2.25 net. [72]

Bailey's Technical Dictionaries Catalogue 1974. Pp. 80. (Folkestone: Bailey Bros. and Swinfen, Ltd., 1975.) 70p. [72]

Ministry of Agriculture, Fisheries and Food. Agricultural Chemicals Approval Scheme: Insecticides Fungicides/Herbicides. 1975 List of Approved Products and their Uses for Farmers and Growers. Pp. 182. (Harpden, Herts: Agricultural Chemicals Approval Organization, Ministry of Agriculture, Fisheries and Food, Plant Pathology Laboratory, 1974.) [102]

The Institution of Gas Engineers. Discussions of the 111th Annual General Meeting, Bristol, 1974. (D931–940). Pp. 151. (London: The Institution of Gas Engineers, 1975.) £1. [102]

Other countries

- Royal Ontario Museum. Life Sciences Occasional Papers, No. 26: Thermal Relations of the Neotropical Frog *Hyla labialis* (Anura: Hylidae). By D. Valdivieso and J. R. Tamsitt. Pp. 12. 60c. Life Sciences Contributions, No. 99: A New Genus of Freshwater Triclad from Tasmania, with Reviews of the Related Genera *Cura* and *Neppia* (Turbellaria: Tricladida). By Ian R. Ball. Pp. 48. \$2. No. 100: A Revision of the Latipinnate Ichthyosaurs of the Lower Jurassic of England (Reptilia: Ophthosauria). By C. McGowan. Pp. 30. \$1.50. No. 102: Fauna and Correlation of the Ravenscrag Formation (Paleocene) of Southwestern Saskatchewan. By Lorin S. Russell. Pp. 52. \$2. A Selected Bibliography on Mercury in the Environment, with Subject Listing. By Susan Robinson and W. B. Scott. Pp. 54. \$1.25. (Toronto: Royal Ontario Museum, 1974.) [271]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Paper 74-9: Trace Element Variations in Porphyry Copper Deposits, Babine Lake Area, B.C. By J. L. Jambor. Pp. v + 30. \$2.50. Paper 74-60: Computer Use in Projects of the Geological Survey of Canada. By Terry Gordon and W. W. Hutchison. Pp. v + 108. \$3. Miscellaneous Report No. 21: Guide to the Geology of Prince Albert National Park—a Story of Hills, Lakes and Beaches. By A. H. Lang. Pp. vii + 40. \$2. Miscellaneous Report No. 22: Guide to the Geology of Elk Island National Park—The Origin of its Hills and other Scenery. By A. H. Lang. Pp. vii + 31. \$1.50. (Ottawa: Information Canada, 1974.) [271]
- CERN — European Organization for Nuclear Research. CERN 74-25: Effet sur la Racine Principale de Vicia Faba de l'Exposition aux Rayons Gamma du ^{60}Co , aux Protons de 600 MeV et aux Neutrons de 14 et de 400 MeV. Par J. M. Laurent. Pp. vi + 122. (Geneva: CERN, 1974.) [281]
- Smithsonian Contributions to Zoology, No. 172: The So-called Cheirodontin Fishes of Central America with Descriptions of Two New Species (Pisces: Characidae). By William L. Fink and Stanley H. Weitzman. Pp. iii + 46. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.05. [281]
- Australia: Commonwealth Scientific and Industrial Research Organization. Division of Food Research—Report of Research, 1973/1974. Pp. 93. (Sydney: CSIRO, Division of Food Research, 1974.) [291]
- Anuario del Observatorio Astronómico de Madrid para 1975. Pp. 414. (Madrid: Observatorio Astronómico, 1974.) 100 pesetas. [301]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tropical Agronomy, 1973/1974. Pp. 168. (Brisbane, Qd.: CSIRO, 1974.) [311]
- Centre National pour l'Exploitation des Océans. Publications du CNEXO, Série: Résultats des Campagnes à la Mer, No. 08—Résultats de la Campagne Mediprod III (13 Juin-2 Juillet, 1972). Pp. 21. (Paris: CNEXO, 1974.) [311]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 234: Evolution of a Middle and Upper Devonian Sequence from a Clastic Coastal Plain-Deltaic Complex into Overlying Carbonate Reef Complexes and Banks, Sturgeon-Mitsue Area, Alberta. By L. F. Jansa and N. R. Fischbuch. Pp. 105. \$5. Memoir 371: Geology of Tetagouche Lakes, Bathurst, and Nepisiguit Falls Map-Areas, New Brunswick, with emphasis on the Tetagouche Group. By Ralph Skinner. Pp. 133 (42 plates). \$8. (Ottawa: Information Canada, 1974.) [32]
- Organization for Economic Co-operation and Development. OECD Informatics Studies No. 7: Information Technology in Local Government: a Survey of the Development of Urban and Regional Informations Systems in Five European Countries. Pp. 168. (Paris: OECD; London: HMSO, 1974.) 20 francs; £2; \$5. [32]
- International Institute of Cellular and Molecular Pathology—Descriptive Booklet. Pp. 199. (Bruxelles: International Institute of Cellular and Molecular Pathology, Avenue Hippocrate, 1975.) [32]
- Saving Our Small World. (Monday Conference on Population, Ecology and Resources.) Pp. 131. (Sydney: The Australian Broadcasting Commission, 1974.) \$2.50. [32]
- Scripps Institution of Oceanography. Annual Report year ending June 30, 1973. Pp. 64. (San Diego: Scripps Institution of Oceanography, University of California, 1974.) [32]
- On Unification, Part 3. By Sakawat Hossain. Pp. 47-72. Supplement. Pp. 14. (Dacca, 5: Sakawat Hossain, 267 Elephant Road, 1972.) [32]
- Marine Science Teaching at the University Level—Report of the Unesco Workshop on University Curricula. (Unesco Technical Papers in Marine Science, No. 19.) Pp. 27. (Paris: Unesco, 1974.) [32]
- New Trends in Integrated Science Teaching: Education of Teachers. Edited by P. E. Richmond. Pp. x + 227. (Paris: The Unesco Press, 1974.) [52]
- Australia: Commonwealth Scientific and Industrial Research Organization. Land Research Series No. 33: Explanatory Notes to the Geomorphological Map of Papua New Guinea. By E. Löffler. Pp. 19 + map (4 sheets). (Melbourne: CSIRO, 1974.) [62]
- Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Animal Health for 1973. Pp. 119. (East Melbourne: CSIRO, 1974.) [62]
- Carnegie Institution of Washington. Report of the President, 1973/1974. Pp. 32. (Washington, DC: Carnegie Institution of Washington, 1975.) [72]
- United States Department of the Interior: Geological Survey. Professional Paper 805: Lower and Lower Middle Devonian Rugose Corals of the Central Great Basin. By C. W. Merriam. Pp. v + 83 + plates 1-25. (Washington, DC: Government Printing Office, 1974.) \$2.85. [72]
- Science Council of Canada. Background Study No. 32: Technology Transfer in Construction. By A. D. Boyd and A. H. Wilson. Pp. 163. (Ottawa: Information Canada, 1974.) \$3.50. [102]
- Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin No. 218: Keweenaw Volcanic Rocks of Michipicoten Island, Lake Superior, Ontario, and Eruptive Centre of Proterozoic Age. By R. N. Annells. Pp. 141 (22 plates). (Ottawa: Information Canada, 1974.) \$5. [102]
- Forschungsberichte des Landes Nordrhein-Westfalen, Nr. 2449: Beziehungen Zwischen der Entropiefreien, der Chemischen und der Rationalen Thermodynamik der Vorgänge. Von Wolfgang Kern, Gunter Weiner und Prof. Josef Meixner. Pp. 39. (Düsseldorf: Westdeutscher Verlag, 1974.) DM.5.20. [112]
- Annual Report of The Experiment Station of the South African Sugar Association, 1973/1974. Pp. 58. (Mount Edgecombe, Natal: Experimental Station of the South African Sugar Association, 1974.) [122]
- Environment Canada: Fisheries and Marine Service Technical Report No. 499: An Automatic Monitor for Detecting and Recording Passage of Transmitter-fitted Fish. By D. G. Pincock, D. McG. Luke, D. W. Church and A. B. Stasko. Pp. vi + 59. (St. Andrews, New Brunswick: Research and Development Directorate Biological Station, 1974.) [172]
- United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Supplement 18, Part 6: Parasite-Subject Catalogue—Subject Headings and Treatment. By Martha L. Walker, Jane D. Rayburn, Judith M. Humphrey and Dorothy B. Segal. Pp. iii + 339. \$2.95. Supplement 19, Part 6: Parasite-Subject Catalogue—Subject Headings and Treatment. By Martha L. Walker, Jane D. Rayburn, Judith M. Humphrey and Dorothy B. Segal. Pp. xv + 672. \$6.05. Supplement 19, Part 1: Authors—A to Z. By Dorothy B. Segal, Judith M. Humphrey, Shirley J. Edwards, Margie D. Kirby, Martha L. Walker, Jane D. Rayburn, Lila R. Crawley and J. M. Podani. Pp. xvii + 783. \$7.35. (Washington, DC: Government Printing Office, 1973 and 1974.) [172]
- Bibliographien des Deutschen Wetterdienstes. Nr. 28: Agrarmeteorologische Bibliographie 1973. Bearbeitet von Maximilian Schneider. Pp. xxvii + 246. (Offenbach a.m.: Selbstverlag des Deutschen Wetterdienstes, 1974.) [182]
- Institute of Radiation Breeding, Ibaraki-ken, Japan: Gamma Field Symposia. No. 11: Chemical Mutagenesis in Micro-organisms and Plants. (Report of Symposium held on July 25 to 26, 1972.) Pp. 96. No. 12: Induced Mutation and Chimera in Woody Plants. (Reports of Symposium held on July 26 to 27, 1973.) Pp. 120. (Ohmiya-machi, Ibaraki-ken: Institute of Radiation Breeding, NIAS MAF, 1972 and 1973.) [182]
- Deutsches Hydrographisches Institut, Hamburg. Jahresbericht 1972/1973. Pp. 158. (Hamburg: Deutsches Hydrographisches Institut, 1974.) [182]
- The Open Universities in South Africa and Academic Freedom, 1957/1974. A Review by the Academic Freedom Committees of the University of Cape Town and the University of the Witwatersrand, Johannesburg. Pp. x + 49. (Cape Town and Johannesburg: Juta and Co., Ltd., 1974.) [192]
- United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Zoology. Special Publication No. 3: Ticks and Tickborne Diseases. I. Genera and Species of Ticks, Part 1: Genera A-G. Pp. 429. Part 2: Genera H-N. Pp. 593. Part 3: Genera O-X. Pp. 329. By Mildred A. Doss, Marion M. Farr, Katharine F. Roach and George Anastos. (Washington, DC: Government Printing Office, 1974.) [192]
- Smithsonian Contributions to Astrophysics. No. 17: Period, Colour, and Luminosity for Cepheid Variables. By Cecilia H. Payne-Gaposchkin. Pp. 10.50 cents. Smithsonian Contributions to Zoology, No. 187: Communication in Red Fox Dyads—a Computer Simulation Study. By Gerald Gene Montgomery. Pp. iii + 30. 95 cents. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) [202]
- United States Department of the Interior: Geological Survey. Professional Paper 866: Tectonic Features of the Precambrian Belt Basin and their Influence on Post-Belt Structures. By Jack E. Harrison, Allan B. Griggs and John D. Wells. Pp. iii + 15. (Washington, DC: Government Printing Office, 1974.) 65 cents. [202]
- A Bibliography of Soviet Sources on Medicine and Public Health in the USSR. Prepared Under Contract by Ms. Lee Parkins. (A Publication of Geographic Health Studies by the John E. Fogarty International Center for Advanced Study in the Health Sciences.) Pp. xix + 235. (Bethesda, Md.: US Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1975. For sale by US Government Printing Office.) \$32.00. [202]
- United States Department of the Interior: Geological Survey. Bulletin No. 1327: The Geologic Story of Canyonlands. By S. W. Lohman. Graphics by John R. Stacy. Pp. ix + 126. (Washington, DC: Government Printing Office, 1974.) \$2.65. [212]
- Energy R&D: Problems and Perspectives. Pp. 243. (Paris: OECD; London: HMSO, 1975.) 35 francs; £3.50; \$8.75. [212]
- Die Akademien der Wissenschaften der Sowjetischen Unionsrepublik: Struktur und Aufgaben Verzeichnis der Institute. Von Wolfgang Kasack. Pp. 134. (Boppard: Harald Boldt Verlag, K.G., 1974.) [242]
- Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIV, No. 10: A New, Unified Interpretation of U Cephei. By Alan H. Batten. Pp. 191-129. (Victoria, BC: Dominion Astrophysical Observatory, 1974.) [242]
- Importancia Biológica y Social de las Reservas Naturales: Estación Experimental de Fauna Silvestre de San Cayetano, Estado de México. Por C. M. Gallegos y W. C. Domínguez. Pp. viii + 90. Los Crocodiles de México (Estudio Comparativo). Por M. S. del Toro. Pp. viii + 70. (México, DF: Instituto Mexicano de Recursos Naturales Renovables, A.C., 1974.) [242]
- The International Nickel Company of Canada, Ltd. 1974 Annual Report. Pp. 32. (Sudbury, Ontario: The International Nickel Company of Canada, Ltd., 1975.) [252]

Person to Person

Amentiferae collections. Anyone willing to collect regularly material for a floral development study of Myricaceae, Juglandaceae and Fagales is asked to contact as soon as possible: Dr Alastair D. Macdonald, Biology Department, Lakehead University, Thunder Bay, Ontario, Canada P7B 5E1.

Newcastle-Bristol. Furnished/unfurnished house or ground-floor flat required central Bristol or Frenchay area from June 1-December 31. For rent or exchange with four-bedroom house in Newcastle for same or shorter period. (Professor J. W. Thompson, Department of Pharmacological Sciences, Medical School, The University, Newcastle upon Tyne NE1 7RU, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

European Organization for Nuclear Research—CERN. CERN 75-1: Calculation of the Neutron-Induced Background in the Gargamelle Neutral Current Search. By W. F. Fry and D. Haidt. Pp. v + 19. (Geneva: CERN, 1975.) [132]

Indian Forest Bulletin (New Series), No. 266: Use of Insecticides for Pest Management in Forests. By V. P. Sharma. Pp. 63. Rs. 3.50; 41p; \$1.26. No. 268: Effect of Treating Conditions on the Anti-Shrink Effect and Bulking Attained in Wood by Urea and Sorbitol Soak Treatments. By S. N. Sharma, N. R. Das, R. C. Bhatnagar and R. C. Lohani. Pp. 19. Rs. 2.10; 24p; 76 US cents. No. 269: Cleavage Properties of Some Indian Timbers. By A. C. Sekhar and A. S. Gulati. Pp. 9. Rs. 2; 30p; 94 US cents. No. 270: Chemical Seasoning of heavy and Refractory Axlewood (*Anogeissus latifolia*) Using Polyethylene Glycol-600. By S. N. Sharma, M. L. Mehra and S. P. Badoni. Pp. 26. Rs. 1.45; 18p; 54 US cents. Indian Forest Leaflet No. 195: Effects of Grain Alignment of Wooden Discs Made from Radial (Quarter-Sawn) and Tangential (Plain Sawn) Planks on the Strength of Structural Joints. By N. J. Masani and K. S. Pruthi. Rs. 2.20; 26p; 80 US cents. (Delhi: Manager of Publications, 1974.) [172]

Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, Bruxelles. Comptes Rendus de Recherches, No. 38: Travaux du Centre de Recherches sur les Maladies Parasitaires des Animaux Domestiques. Pp. 154. (Bruxelles: IRSIA, 1974.) [172]